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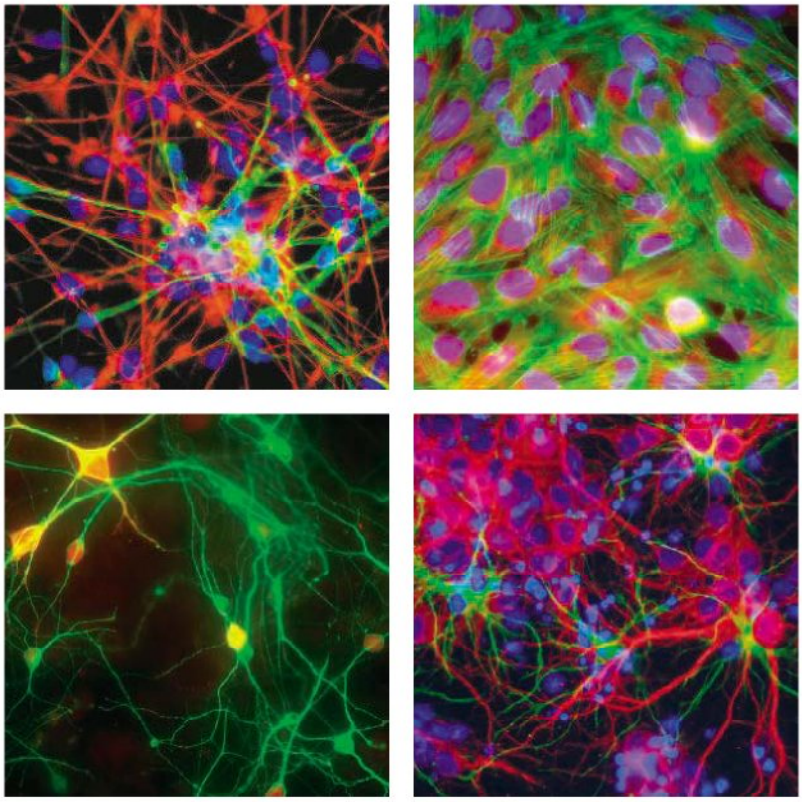

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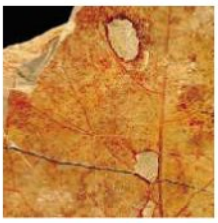

COVER Fossil leaf from the earliest Eocene (~55.5 million years ago) of the Bighorn Basin, Wyoming. The beginning of the Eocene was characterized by rapid global warming after a huge release of carbon into the atmosphere and ocean. Plant fossils described on page 993 document rapid, continental-scale changes in the geographic ranges of plants coincident with the warming. [Photo: S. Wing]

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DEPARTMENTS

NEWS OF THE WEEK

NEWS FOCUS

960

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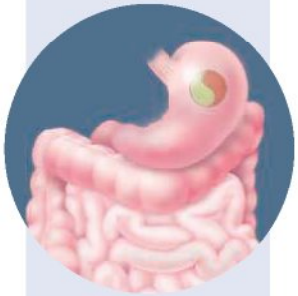

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Systems Biology — DNA Amplification

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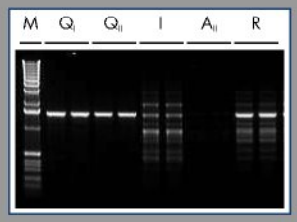

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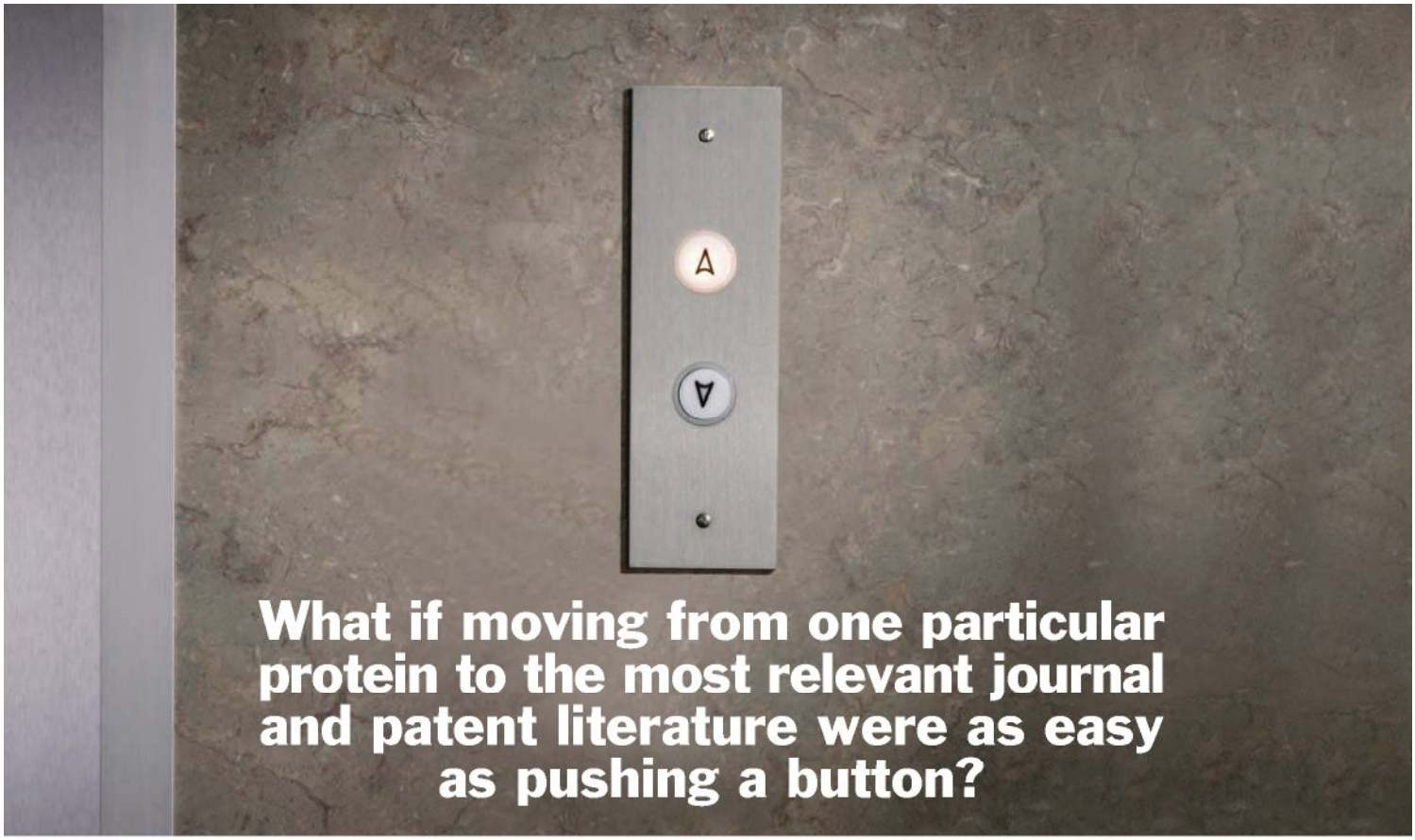

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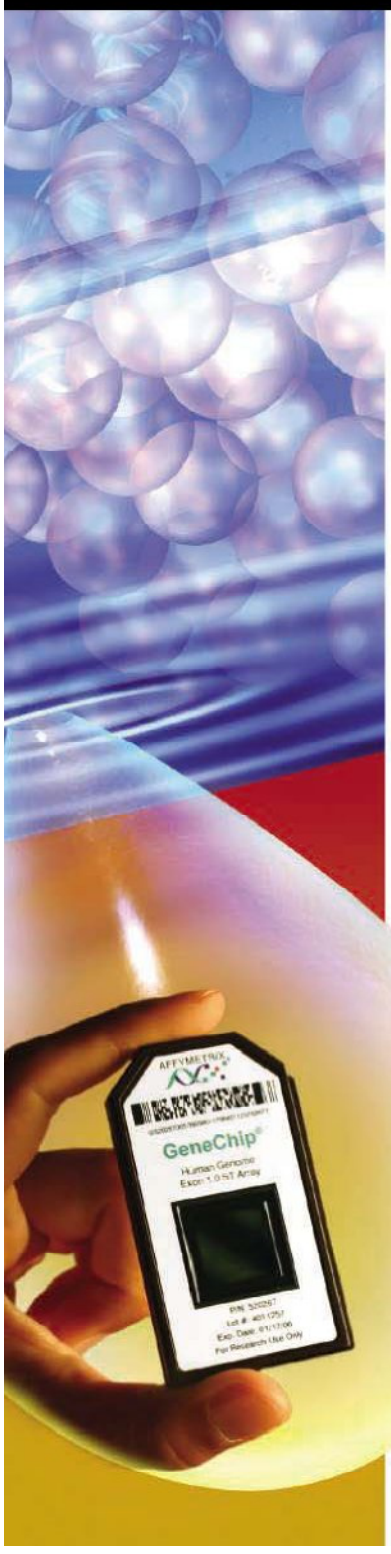
Opt for innovation



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Icebergs may have accelerated changes in penguin DNA.

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Career Development Center: Lab Dynamics—Doing Everyone Else's Job *C. Cohen and S. Cohen*
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Career Development Center: How to Get a Grant *GrantDoctor*
The GrantDoctor reviews some of the basic principles of good grantsmanship.

Europe: The European Young Investigator Awards—Meet the 2005 Laureates *E. Pain and A. Forde*
Next Wave talks with some of the 2005 winners about their career paths and what made them successful.

GrantsNet: International Grants and Fellowships Index *Next Wave Staff*
Get the latest listing of funding opportunities from Europe, Asia, and the Americas.

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A scientist recently profiled in Next Wave gives credit where credit is due.

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Review: Mitochondrial Genetics of Aging—Intergenomic Conflict Resolution *D. M. Rand*
Understanding biochemical and genetic conflicts between mitochondria and nucleus sheds light on aging.

News Focus: Cancer Connection *M. Leslie*
Tumor-fighter throttles life-extending protein SIRT1.

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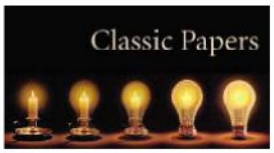

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Connections Map: G Protein Signaling in the Regulation of Rice Seed Germination *S. M. Assmann*
G proteins are implicated in the regulation of rice seed germination by the plant hormones gibberellic acid and abscisic acid.

Connections Map: G Protein Regulation of Disease Resistance During Infection of Rice with Rice Blast Fungus *S. M. Assmann*
Just as in animals, plant responses to pathogenic agents involve G protein signaling.

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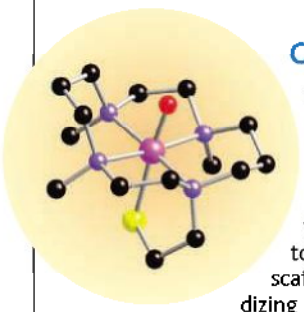


Oxo Above, Sulfur Below

Sulfur coordination from cysteine to iron likely affects the selectivity of hydrocarbon oxidation by cytochrome P450 enzymes. However, small model compounds that could offer more details on the reaction mechanism have been hard to construct, because without the protein scaffold, sulfur ligands are unstable in an oxidizing environment. **Bukowski *et al.*** (p. 1000, published online 27 October) have prepared an iron complex with a modified cyclotetradecane ligand, which like heme has four coordinating nitrogen atoms, but also bears a pendant thiolate group rigidly positioned near the metal. Mössbauer and x-ray absorption spectroscopy confirmed that this molecule can form an Fe=O bond at low temperature, while retaining the coordinated sulfur opposite the oxo group. The sulfur-bound iron oxo favored one-electron over two-electron oxidation chemistry relative to an analogous compound in which the sulfur ligand was absent.

Looking Through HOOPs

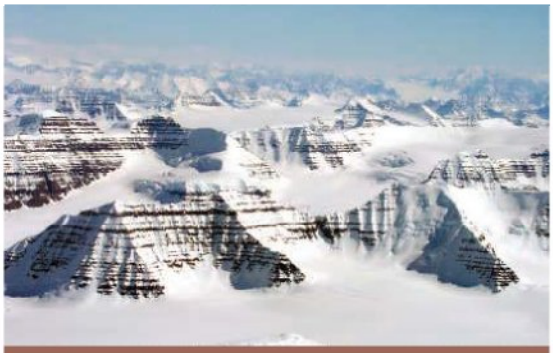
The molecular trigger for visual response is a light-induced cis-to-trans isomerization in the retinal chromophore of rhodopsin that occurs in less than 1 picosecond. **Kukura *et al.*** (p. 1006; see the Perspective by **Champion**) have used femtosecond-stimulated Raman spectroscopy to discern which atoms move when in this process. Their technique offers sufficient simultaneous time and frequency resolution to monitor the coherent spectral features due to hydrogen out-of-plane (HOOP) bending motions around the isomerizing alkene group. By modeling the data, they find evidence for a pathway of rapid (<200 femtoseconds) electronic relaxation, followed by twisting of a distorted retinal backbone to the relaxed trans structure over the ensuing 800 femtoseconds.

Transitional Forcing

During the mid-Pleistocene, the characteristic length of glacial cycles changed from 41,000 to 100,000 years. There has been much speculation about what might have caused that transition, and about the respective roles of high-latitude and low-latitude processes. **Medina-Elizalde and Lea** (p. 1009, published online 13 October) reconstructed a history of sea surface temperature (SST) in the western equatorial Pacific warm pool for the time interval between 1.3 million and 450,000 years before the present. The cyclicity of SST variability shifted from 41,000 to 100,000 years at the mid-Pleistocene transition, and throughout this transition, changes in tropical SSTs preceded changes in ice volume. The authors conclude that atmospheric greenhouse forcing was the cause of the switch in climate periodicities at this time.

Climate Change and Ancient Plant Ranges

Using a plant fossil assemblage from Wyoming, **Wing *et al.*** (p. 993; see the cover) show that global warming at the Paleocene-Eocene boundary (55.8 million years ago) caused rapid change in the geographic ranges of plant species. These range shifts were similar in rate and magnitude to climate-induced change in more recent, postglacial floras. Such short-term ecological change (<10,000 years) has seldom been shown in deep-time records because it is difficult to resolve transient events. The assemblage shows "individualistic" response of species to climate change (similar to conclusions from studies of quaternary pollen records), and that the "stasis" in species composition seen in deep-time records can mask dramatic, geologically short-lived events.



Bigger in the Middle

Rapid thinning is now occurring along the perimeter of the Greenland Ice Sheet, but the response of the interior has been more difficult to determine precisely. **Johannessen *et al.*** (p. 1013, published online 20 October) have compiled a vast set of ice sheet elevations (45 million points) from satellite observations from 1992 to 2003. The expansive interior of the ice sheet is increasing in thickness by an average of around 5 centimeters per year, driven mostly by increasing rates of snow accumulation. The authors suggest that this growth is the result of the North Atlantic Oscillation on winter precipitation. This effect must be considered carefully when predicting ice sheet mass balance changes, because the behavior of the North Atlantic Oscillation is also thought to depend on global warming.

Piece by Piece

The electrochemically driven assembly of oligomers from different thiophene monomers on an iodine-covered gold surface has been visualized by **Sakaguchi *et al.*** (p. 1002) with the scanning tunneling microscope (STM). The polymers are grown on the surface from the monomers in solution by applying voltage pulses to the substrate. The homopolymers formed from 3-octyloxo-4-methylthiophene have a lower energy gap and show broader features in the STM images than do those from 3-octyl-4-methylthiophene. In this way, the different types of copolymer strands formed at the surface can be distinguished.

Dueling Hunger Hormones?

Ghrelin, a circulating peptide hormone produced in the stomach, has attracted much attention because of its stimulatory effect on food intake, but the effect of ghrelin may represent only half of the story. Using a bioinformatics approach, **Zhang *et al.*** (p. 996; see the Perspective by **Nogueiras and Tschöp**) show that *ghrelin* encodes a second peptide hormone that is processed from the same protein precursor as ghrelin. In rodents, a synthetic version of this hormone, obestatin, has the opposite physiological effect as ghrelin—it suppresses food intake. Obestatin mediates its actions through an orphan G protein–coupled receptor, GPR39, which shares sequences with, but is distinct from, the receptor targeted by ghrelin.

CONTINUED ON PAGE 941

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Modeling Complexity

Simple models are often insufficient for predicting or explaining complex systems such as ecosystems or financial markets, but complex, mechanistic models can be difficult to test and cannot be fully analyzed mathematically. **Grimm *et al.*** (p. 987) review several recent advances in simulation modeling in an approach they call pattern-oriented modeling, a general strategy for designing and developing explanatory models of complex systems. Pattern-oriented modeling can predict multiple observed ecological patterns at different levels of organization. This approach can be used to distinguish among alternative model structures, to focus on the most important parameters, and to simplify models when possible.

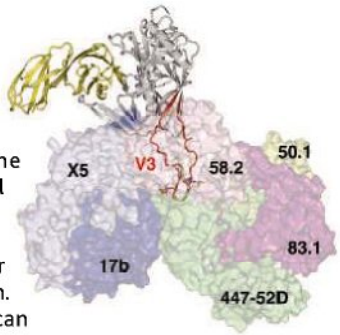
Mainly a Cultural Legacy

Neither archaeological nor modern DNA sequence data have resolved whether modern Europeans are descended from paleolithic communities inhabiting the continent for 40,000 years, or from Neolithic farmers who arrived in Europe after the end of the most recent glaciation 10,000 years ago. **Haak *et al.*** (p. 1016; see the news story by **Balter**) present mitochondrial DNA sequence data derived from 7500-year-old Neolithic human remains excavated from sites in Central Europe to explore the extent to which early farmers generated the present-day genetic profile of Europe. The presence of sequences now rare in modern Europeans suggests that early Neolithic farmers have left little genetic legacy, and that their impact was largely cultural.

Targeting TNF-α Interactions

The proinflammatory cytokine, tumor necrosis factor–α (TNF-α), plays a role in diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis. TNF-α forms a homotrimer that binds to the TNF receptor to activate inflammatory responses. Although antibodies against TNF-α or soluble versions of the receptor are therapeutically effective, rationally designed small-molecule drugs that target protein-protein interactions would be useful. **He *et al.*** (p. 1022) report on a small-molecule inhibitor that functions by dissociating the TNF-α trimer. The inhibitor binds to the intact biologically active trimer, accelerates subunit dissociation, and forms a complex with a dimer of TNF-α subunits.

Detailed View of the HIV Spike



The human immunodeficiency virus (HIV) envelope spike contains three gp120 glycoproteins that promote viral entry into cells. Structures of gp120 unliganded and bound to CD4 receptor have provided important insights but have lacked the immunodominant third variable region (V3) critical for coreceptor binding. **Huang *et al.*** (p. 1025) determined the structure of V3 in the context of an HIV-1 gp120 core complexed to the CD4 receptor and to the X5 antibody at 3.5 angstrom resolution. The structure provides a rationale for how V3 can serve its dual roles in neutralization and HIV entry.

CREDIT: HUANG *ET AL.*

A Time to Grow, A Time to Crop

Barley is a very adaptable grain crop that can be grown from the Arctic Circle to subequatorial near desert regions. Part of barley's success derives from its diverse strains that have various responses to changes in photoperiod. **Turner *et al.*** (p. 1031) have now identified the *Ppd-H1* gene of barley and find that it participates in the coordinate regulation of flowering by circadian clocks and seasonal photoperiod. A spring variety of barley shows reduced photoperiod response caused by a mutation in this gene that delays its flowering. Instead, the plant accumulates the vegetative mass required to produce more grain.

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Biodiversity Science Evolves

The planet's biodiversity is increasingly threatened by human activities. We have heard this before, and the global mantra to stop the damage has forged numerous international panels and agreements over the past 15 years. Yet despite these efforts to ensure biodiversity conservation, we have witnessed extensive population extinctions and massive deforestation and fragmentation of natural habitats, and we may even see the geographic contraction of major ecosystems, such as the tropical rainforest in its northernmost distribution in the Americas. Our quantification of species extinction is poor, yet we do know that the number of threatened species, including the most charismatic animals, is considerable. For example, 25% of all the mammals on the planet are endangered. Obviously, there continue to be problems with enforcing conservation in the face of social and economic growth in industrialized and developing countries.

This week, DIVERSITAS, the international program on biodiversity science, is holding its first open science conference in Oaxaca, Mexico, to discuss why the challenge of biodiversity conservation—arguably one of the biggest challenges facing modern society—remains so formidable, and how the international scientific community can be moved into action to address this problem. The timing of this conference is appropriate: It follows the Millennium Ecosystem Assessment, released in May 2005, which provides a comprehensive analysis of past and future trends in the state of ecosystems and discusses what information is necessary to inform policy decisions on conservation.

Increasingly robust databases on species distribution and analytical tools such as remote-sensing and climate change models have allowed us to make substantial progress toward understanding biodiversity distribution and rates of change. Likewise, we have begun to explore synergies between the drivers of biodiversity change, and there is a greater understanding of the relationships between biodiversity and ecosystem functioning. However, although compelling, these findings and knowledge are still being interpreted in isolation from one another, and this has perhaps been one of the major problems in achieving the goals of protecting biodiversity. The biodiversity scientific community is fragmented among types of ecosystems (terrestrial, freshwater, and marine); types of organisms (such as vertebrates, invertebrates, plants, and microbes); and, perhaps most critically, among disciplines (taxonomy, molecular biology, ecology, and socioeconomic sciences). Consequently, biodiversity science has been undervalued by the policy sectors.

As an important move toward integration, the DIVERSITAS conference, "Integrating Biodiversity Science for Human Well-Being," is providing a venue for researchers and students from different disciplines, as well as policy-makers, to assess the current strengths and weaknesses of biodiversity science and its main future challenges. The scientific challenges are enormous. We need many new technologies: molecular and bioinformatic tools to examine Earth's biodiversity; a coordinated observation system and standardized methods to monitor biodiversity; integrated analyses and models of social, ecological, and evolutionary processes to predict future biodiversity changes; and large-scale experimental facilities and new models to understand and predict the multiple effects of biodiversity changes on ecosystem services and human societies. At the same time, new approaches are needed to optimize the multiple uses of biodiversity in ways that consider tradeoffs and conflicts between conservation and development options and that incorporate the ethical dimensions of biodiversity conservation. Conservation in human-dominated landscapes as well as protected areas (only 10 to 11% of the land surface) will require that it become a socially and economically attractive activity that takes into consideration local inhabitants and landowners. This will require new economic approaches to ensure that rural inhabitants are compensated when they opt to conserve their land. The Costa Rican experience of sustained programs of payment to farmers as compensation for setting aside forest for biodiversity conservation and ecosystem services is a promising example.

For biodiversity science to progress so that it produces socially relevant knowledge—in the sense that it can help society to better understand and capitalize on the value of biodiversity—it must evolve. There is an urgent need to integrate biological and social disciplines in order to generate reliable recommendations for society and to incorporate biodiversity conservation and use into mainstream policy worldwide. We need unity in diversity.

Rodolfo Dirzo and Michel Loreau

Rodolfo Dirzo is in the Department of Biological Sciences, Stanford University, Stanford, California, and is vice-chair of DIVERSITAS. Michel Loreau is in the Department of Biology, McGill University, Canada, and is chair of DIVERSITAS.

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EDITORS' CHOICE

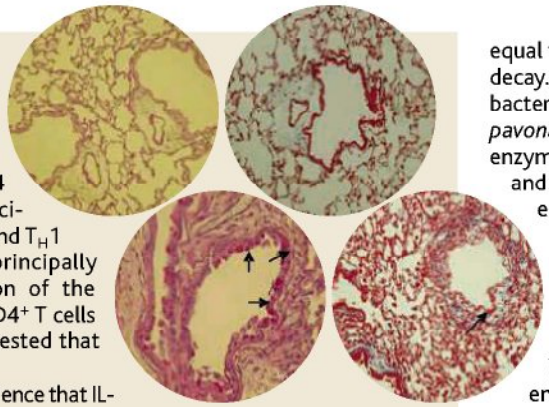
edited by Gilbert Chin

IMMUNOLOGY

An Inflammatory Lineage

Helper CD4$^+$ T (T_H) cells are traditionally divided into two principal lineages: interleukin-4 (IL-4)/IL-5–producing T_H2 cells, which are associated with allergic and antiparasitic responses, and T_H1 cells that produce inflammatory cytokines, principally interferon-γ (INF-γ). However, the expression of the cytokine IL-17 by a relatively small subset of CD4$^+$ T cells and its association with inflammation has suggested that this may define a T_H1 sublineage.

Now, Harrington *et al.* and Park *et al.* provide evidence that IL-17–producing CD4$^+$ T cells may represent a distinct T-helper population altogether, the development of which is coordinately regulated with those of T_H1 and T_H2 cells. Both studies confirmed the dependence of IL-17 expression on signaling through the receptor for the cytokine IL-23 and demonstrated that this was independent of the signals and transcriptional pathways responsible for IFN-γ and IL-4 production. Furthermore, both of these T-helper cytokines were found to inhibit IL-17 expression in naïve T cells—as opposed to differentiated IL-17$^+$ T cells—suggesting a dominant role in cross-regulation during early T cell priming. Given the clear association of IL-17 with tissue inflammatory responses, the strict management of T_H17 cell differentiation may represent a central checkpoint in preventing immune pathologies such as those seen in autoimmune diseases. — SJS



Lung inflammation induced by overexpression of IL-17 (lower row), with increases in mucus production (arrows, left) and collagen deposition (arrow, right) in bronchioles.

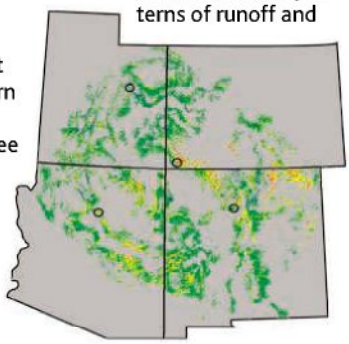
Nat. Immunol. 6, 1123; 1133 (2005).

CLIMATE SCIENCE

Warmer and Drier

One effect that is expected to accompany global warming is the occurrence of more intense and more frequent droughts. Although it is known that protracted drought increases tree mortality, the response of forests on regional or continental scales to the kind of warmer drought that may occur in the future is poorly understood.

Breshears *et al.* examined the impact of recent drought on piñon pine trees in western North America, focusing on the relationships between tree die-off, temperature, and rainfall. They found that the 2000–2003 drought was not as dry as the previous one of 1953–1956, but that it occurred during a warmer period and hence might illustrate drought effects in the future. Their analysis shows that the recent drought caused a rapid regional-scale loss of overstory trees mainly due to infestation by bark beetles, outbreaks of which are commonly caused by water stress; whereas the 1950s drought affected mainly older trees, the 2000s drought killed trees of all ages. Similar widespread drought in this century could cause large changes in carbon storage and dynamics, in fluxes of near-ground solar radiation, and in patterns of runoff and erosion, as well as alter microclimate feedbacks between the land and atmosphere and reduce the production of piñon nuts, an important food source for a number of species of birds, small mammals, and local people. — HJS



Changes in the normalized difference vegetation index (green, no change; red, largest decrease) in the southwestern US.

Proc. Natl. Acad. Sci. U.S.A. 102, 15144 (2005).

BIOCHEMISTRY

New Activity, Old Enzyme

Ever since we realized that chemicals introduced into the environment for the control of agricultural pests can persist for uncomfortably long periods, there has been an interest in microbes that are able to adapt to living off of (metabolizing) these synthetic carbon sources. In the case of the nematocide 1,3-dichloropropene, its degradation product, *trans*-3-chloroacrylic acid, undergoes hydrolytic decomposition with a half-life of 24,000 years at 19°C, roughly equal to the half-life for ^{239}Pu decay. Fortunately, the soil bacterium *Pseudomonas pavonaceae* expresses the enzyme CaaD, which Horvat and Wolfenden show accelerates hydrolysis, yielding malonate semialdehyde through addition of water and loss of HCl, by a factor of 10^{12}. They argue that this impressive rate enhancement is due largely to chemical transformations taking place in the active site (as opposed to substrate binding or product release) and that CaaD appears to be a considerably more proficient enzyme than its structural cousin 4-oxalocrotonate tautomerase, all of which provides support for the proposal that the degradation of 3-chloro-acrylate may be a recently acquired activity of a relatively ancient and catalytically sophisticated enzyme. — GJC

Proc. Natl. Acad. Sci. U.S.A. 102, 16199 (2005).

GEOCHEMISTRY

Reversing Crystal Growth

Much of the chemistry and dynamics of Earth's surface depends on the dissolution of minerals: It determines the composition of soils, rivers, and oceans and affects the amounts of major gases, such as CO_2, in the atmosphere. Rapid dissolution weakens rocks, facilitating erosion, and dissolution and corrosion are critical in evaluating the performance of engineered structures. Various data have implied that the dissolution rates of many minerals are complex functions, depending subtly on interacting waters, for example.

Dove *et al.* show, both theoretically and through experi-

CONTINUED ON PAGE 947

CREDITS: (TOP) PARK *ET AL.*, *NAT. IMMUNOL.* 6, 1133 (2005); (BOTTOM) BRESHEARS *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* 102, 15144 (2005)

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ments, that for quartz, and likely for other silicate minerals, well-developed theories of crystal nucleation and growth can be used to understand dissolution. Nucleation theory involves four parameters: temperature, oversaturation, and two parameters that describe the energy and kinetics associated with a step on a growing crystal. The authors derive the analogous equations for dissolution at dislocations and vacancies, and show that the theory fits well with experimental data for quartz, feldspar, and a common clay mineral, dissolving in waters under a range of pH and salt conditions. If the result holds across a full range of minerals, it would allow the prediction of dissolution and corrosion under a variety of conditions and temperatures. — BH

Proc. Natl. Acad. Sci. U.S.A. **102**, 15357 (2005).

Ternary A/B/C blends (blue/red/green)

APPLIED PHYSICS

Patterns of Light

Polymers have found use in the fabrication of optoelectronic and magnetic devices and as inexpensive, flexible, and lightweight templating materials. Patterns are created through the solvent or by thermally driven phase separation of a blend of homopolymers or block copolymers. One problem with using homopolymers is that it is difficult to create large areas that are defect-free yet retain precise patterning on a much smaller scale. Block copolymers are better for achieving this, but changes in the pattern can require the synthesis of a new copolymer.

Travasso *et al.* describe an alternative method for creating materials that are spatially patterned on the submicrometer scale and are defect-free on the millimeter to centimeter scale. They consider a ternary A/B/C blend of immiscible polymers. Polymers A and B are chosen so that the extent to which they interact or separate can be tuned by exposure to light. Initially, a uniform light source is used to create a homogenous mixture of A and B. By rastering over the sample with a higher-intensity secondary beam, defects in the local pattern can be annealed out. Polymer C is chosen to migrate to areas illuminated by the higher-intensity light. Thus, it is possible to write regions of polymer C onto a spatially patterned AB film. — MSL

Langmuir 10.1021/la052511a (2005).

HIGHLIGHTED IN *SCIENCE*'S SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

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Bacterial Pheromone for Sex and Abstinence

Bacteria can transfer DNA through conjugation, and the transfer of these extrachromosomal elements contributes to virulence and antibiotic resistance. Chandler *et al.* report that in *Enterococcus faecalis*, a mammalian pathogen, the same pheromone that stimulates a donor bacterium to initiate conjugation with a plasmid-free recipient is also produced by the donor itself and regulates its sensitivity to the recipient-produced pheromone. The bacterial chromosome encodes the pheromone (cCF10), so both donor and recipient can produce this molecule; to prevent conjugation with other donors, donor cells have two mechanisms for suppressing the response to the endogenously produced pheromone. One of the conjugation inhibitors is a secreted inhibitor protein, iCF10, which binds and sequesters secreted cCF10, and another is the membrane protein PrgY, which degrades or binds cCF10 as it is released. Using mutant bacterial strains that lacked functional cCF10, Chandler *et al.* show that cCF10 produced by the donor cells stimulates the production of iCF10. Donor cells grown in human plasma or in vivo also produce the plasmid-encoded aggregation factor Asc10, which contributes to cellular invasion and virulence of the bacteria. Albumin was identified as the plasma protein that bound iCF10, thereby shifting the balance between iCF10 and cCF10, allowing self-induction of the conjugation genes, including the one encoding Asc10. — NRG

Proc. Natl. Acad. Sci. U.S.A. **102**, 15617 (2005).

CREDITS: TRAVASSO *ET AL.*, *LANGMUIR* 10.1021/LA052511A (2005)

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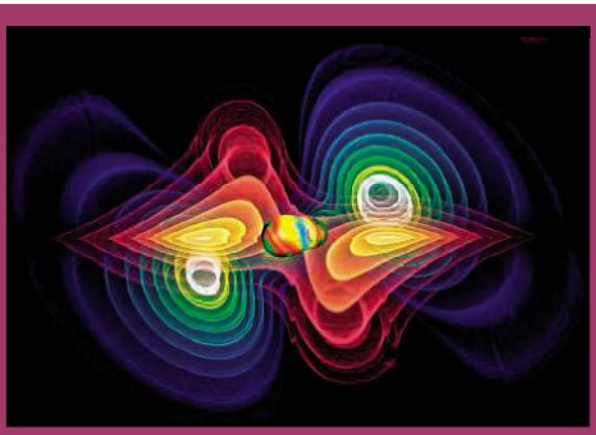


EDUCATION

Brush Up Your Einstein

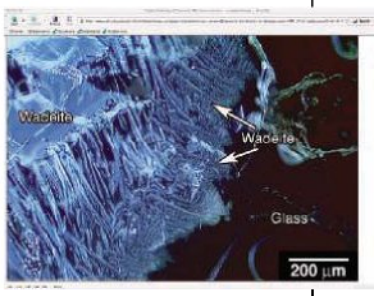
The 100th anniversary celebrations for Einstein's special theory of relativity are winding down, but you can still bone up on his ideas at the tutorial Einstein Online from the Max Planck Institute for Gravitational Physics in Potsdam, Germany. The Elementary Einstein section gives beginners a quick tour of special relativity—which posited that time and length vary with speed—and general relativity, which added gravity to the picture. The section also probes some of relativity's consequences and predictions, including gravitational waves, undulating spacetime distortions that researchers haven't yet measured directly. (Above, a simulation shows gravitational waves from two merging black holes.) Spotlights on Relativity tackles questions such as whether the universe contains more than three dimensions, a prediction of string theory. The site's glossary defines more than 250 relativistic terms.

www.einstein-online.info/en

IMAGES

Out of the Kiln

Ceramics may bring to mind those lopsided pots and lumpy plates children make in grade school. But so-called advanced ceramics show up in everything from armor for military vehicles to bone implants. This site* from the University of Dayton in Ohio can help students and researchers understand how the fine structure of these newfangled ceramics determines their properties. The Digital Library of Ceramic Microstructures houses more than 900 close-ups of materials such as this wadeite crystal from a flat-panel display (right). The collection also includes examples marred by corrosion, oxidation, or other damaging processes, helping users grasp how the materials wear. A similar library† from the University of Cambridge in the U.K. stashes nearly 800 images of ceramics, metals, foams, and other stuff. For materials science teachers, the site also offers a host of lessons and lab exercises.



* www.udri.udayton.edu/dlcm/Home.asp
† www.msm.cam.ac.uk/doitpoms

FUN

From Earth to the Moon

The NASA software World Wind can whisk you on a virtual trip to anywhere on Earth (NetWatch, 13 May, p. 933). Now, you can tour the moon with a new version of the program that uses data from the Clementine spacecraft, which mapped the lunar surface in 1994.

worldwind.arc.nasa.gov/moon.html

RESOURCES

Keeping Tabs on Rare Diseases

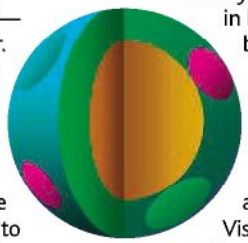
Doctors have recorded only about 100 cases of Kabuki syndrome, a congenital form of mental retardation. The condition got its name because patients' facial features—including arched eyebrows and elongated eye openings—resemble the makeup style in Japanese Kabuki theater. At Orpha.Net, you can find out more about Kabuki syndrome and hundreds of other disorders affecting no more than one person out of 2000. Researchers can search for information on a particular disease and pull up a description of the symptoms and underlying cause. The entries also record orphan drugs, treatments for rare diseases that are unprofitable to manufacture, and link to research projects and clinical trials around the world. The site, which has information in six languages, is sponsored by the European Commission, the French government, and other organizations.

www.orpha.net

EDUCATION

Sick at Heart

Looking for a tutorial on the role of fat-ferrying molecules such as high-density lipoprotein (below) in inflammation? Curious about how hypertension fosters kidney disease? These sites from the Baylor College of Medicine in Houston, Texas, offer a wealth of information about high blood pressure and atherosclerosis. Although aimed at doctors, both can help researchers and students bolster their knowledge of these heartbreakers. For example, Lipids Online* features more than 30 slide shows on topics such as metabolic syndrome, a collection of symptoms including out-of-whack lipid levels and excess abdominal fat that promotes heart attacks and strokes. Visitors can also watch videos of lectures by experts in the field or read commentaries on new findings. At Hypertension Online,† you can peruse updates on the latest drug trials and screen an animation that illustrates how high blood pressure injures the heart.



* www.lipidsonline.org
† www.hypertensiononline.org

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This Week

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A failed black hole

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Russia's radioactive legacy

PUBLIC HEALTH

Pandemic or Not, Experts Welcome Bush Flu Plan

The Bush Administration's proposed flu plan, calling for $7.1 billion to help prepare the nation for a deadly influenza pandemic, is generally winning plaudits from public health experts—but not necessarily because they think a pandemic is imminent. Even if no such disaster materializes, they say, the plan will finance a much-needed overhaul of the nation's regular flu vaccine infrastructure.

When he announced the initiative last week, President George W. Bush noted growing concerns that the H5N1 avian influenza now spreading west from Asia could acquire the ability to be transmitted from human to human. In caveats sometimes lost in general press accounts, Bush and other officials emphasized that H5N1 might not morph into a pandemic strain. A plan is needed, they say, to combat the emergence of any superstrain of human influenza, an event that has happened three times since 1900 and that many think is inevitable in the next few years.

The biggest chunk of the money, $2.8 billion, would be spent on what Bush called a "crash program" to speed cell-based vaccine technology. The goal is to be able by 2010 to manufacture a new vaccine for all Americans within 6 months should a pandemic strike. Another $2.5 billion would be used to stockpile existing vaccines and antiflu drugs. The two other components of the strategy are global surveillance and helping federal and state agencies prepare (see table). The funds would be appropriated all at once but spent over several years.

The existing method for manufacturing flu vaccines is outmoded and slow. Virus used to make flu vaccine is grown in eggs, which have to be ordered in advance, and the entire process takes 9 months. The Administration's plan aims to accelerate the production of flu vaccines by growing seed viruses in cell culture instead of eggs. A half-dozen companies are working on cell-based flu vaccines, and one, Sanofi Pasteur, has already received $97 million from the Department of Health and Human Services (HHS). Bringing them to market and building capacity to make pandemic vaccine for all Americans will take 5 years, the Bush plan says. The challenges involve finding a cell line in which the virus grows well and optimizing it for growing high yields in 100,000-liter fermenters. "A lot of it is empirical," says Gary Nabel of the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. Any vaccine would also have to go through clinical trials for safety and efficacy, and the production process must meet regulatory standards.

Although "it's arguable" whether cell-based technology will shave much off the production time, it will allow "surge capacity" to make larger quantities, says Bruce Gellin, director of the National Vaccine Program Office at HHS, because companies won't be limited by the available supply of eggs. The target is 600 million vaccine doses, two per person.

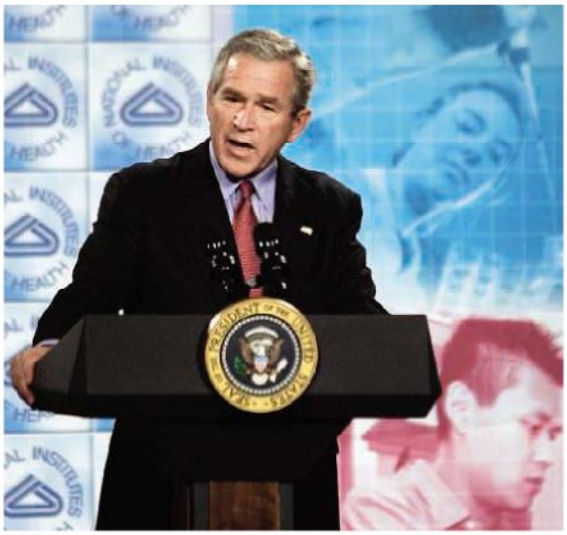
The plan also calls for stockpiling available vaccines and drugs. The government has already funded two companies to manufacture an experimental human H5N1 vaccine. Depending on how much the virus changes, this vaccine might offer some protection should H5N1 acquire the ability to infect people easily. About $1.5 billion is slated for HHS to buy 40 million doses (enough for 20 million people) by 2009 and for the Defense Department to buy vaccine as well. Bush also wants Congress to pass legislation to shield vaccine companies from lawsuits. Some Democrats oppose that step, but "you will never get companies to make hundreds of millions of doses" of vaccine without it, says immunologist Paul Offit of the University of Pennsylvania.

Another $1 billion would buy enough of the antiviral drugs Tamiflu (oseltamivir) and Relenza (zanamivir) for 25% of the population. It is unclear how well Tamiflu would work against H5N1, HHS Secretary Michael Leavitt notes, but it is the only stopgap measure until a vaccine is ready. Similarly, the 25% figure is arbitrary—"pulled out of a hat," says modeler Ira Longini of Emory University in Atlanta, Georgia. However, he says, because only one-third of the population would probably get sick overall, "for purely therapeutic use, 25% would probably be enough." The Infectious Disease Society of America in Alexandria, Virginia, has recommended a stockpile covering 40% of the population.

Many other countries plan to stockpile Tamiflu as well, and it's unclear whether Roche, the only manufacturer, can meet demand. Some lawmakers are calling for Roche to allow other companies to license its Tamiflu technology. Roche said last month it will work with other companies to meet the ▶

Bush's Pandemic Flu Proposal

Cell-culture vaccine manufacturing	$2800 million
Purchasing influenza vaccines	$1519 million
Stockpiling antivirals	$1029 million
Research on new antivirals and vaccines	$800 million
Pandemic preparedness (excluding states)	$544 million
Helping countries detect and contain outbreaks	$251 million
State pandemic plans	$100 million
Other	$9.4 million
TOTAL	**$7.137 billion**



Precautionary principle. President Bush is calling for $7.1 billion to prepare the nation to deal with a possible influenza pandemic, which some experts think is inevitable.

CREDITS: TOP TO BOTTOM): SO...RCE: WHITE HO...SE; EVAN VUCCI/AP PHOTO

Outmoded. Existing methods to make flu vaccine, which involve growing the virus in eggs, are too slow; the plan would support a cell-based alternative.

orders. It says it can fill the U.S. order by summer 2007 and plans to build its first U.S. plant.

Another $251 million will be spent on helping other countries build their capacity to detect and respond to outbreaks. And $644 million will help federal agencies and states prepare.

A separate, 396-page HHS plan, released on 2 November, describes the country's broad public health strategy and spells out who would receive scarce supplies of vaccines and antivirals. Health care workers, the elderly, others at high risk for influenza, and pregnant women would be among the first in line. If certain groups, such as young adults, prove to be more vulnerable to a pandemic strain, the list would be revised, says epidemiologist Arnold Monto of the University of Michigan, Ann Arbor, who advised HHS in developing the plan. The plan also updates draft scenarios, released in August 2004, that predict health care costs alone to the United States of $181 billion for a moderate pandemic. In a severe pandemic, 10 million Americans could be hospitalized, and 1.9 million could die.

The Administration's plan quickly drew congressional fire. In several hearings, lawmakers complained that it would provide insufficient money to states, which are expected to help pay for the antiviral drugs. Others have expressed concerned that the Department of Homeland Security will lead the response, rather than HHS, which has the appropriate public health expertise.

The plan arrives as some experts are questioning whether the likelihood of a devastating pandemic is being exaggerated. Offit, for example, suggests that H5N1, which has sickened 120 people over the past 8 years and killed about half, would have spread from human to human by now if it was going to happen. But he and others are praising the Bush plan anyway because it will help reduce the toll from seasonal influenza. "We're seduced into this tsunami mentality," Offit says. But if you add up annual deaths from influenza, he says the numbers quickly approach pandemic estimates. Longini agrees: "I'm glad all this is happening, but not because of pandemic flu."

–JOCELYN KAISER

HURRICANE KATRINA

Levees Came Up Short, Researchers Tell Congress

When the levees protecting New Orleans gave way under the onslaught of Hurricane Katrina, the most common explanation at the time was that they simply weren't built to withstand a storm of such ferocity. But several teams of engineers told a Senate panel last week that poor design or construction bears much of the blame.

The preliminary reports, from research teams supported by the National Science Foundation (NSF), the American Society of Civil Engineers, and the state of Louisiana, paint a clear picture of how the city's vital flood-protection system failed miserably. "If the levees had done what they were designed to do, a lot of the flooding would not have happened," said civil engineer Raymond Seed of the University of California, Berkeley.

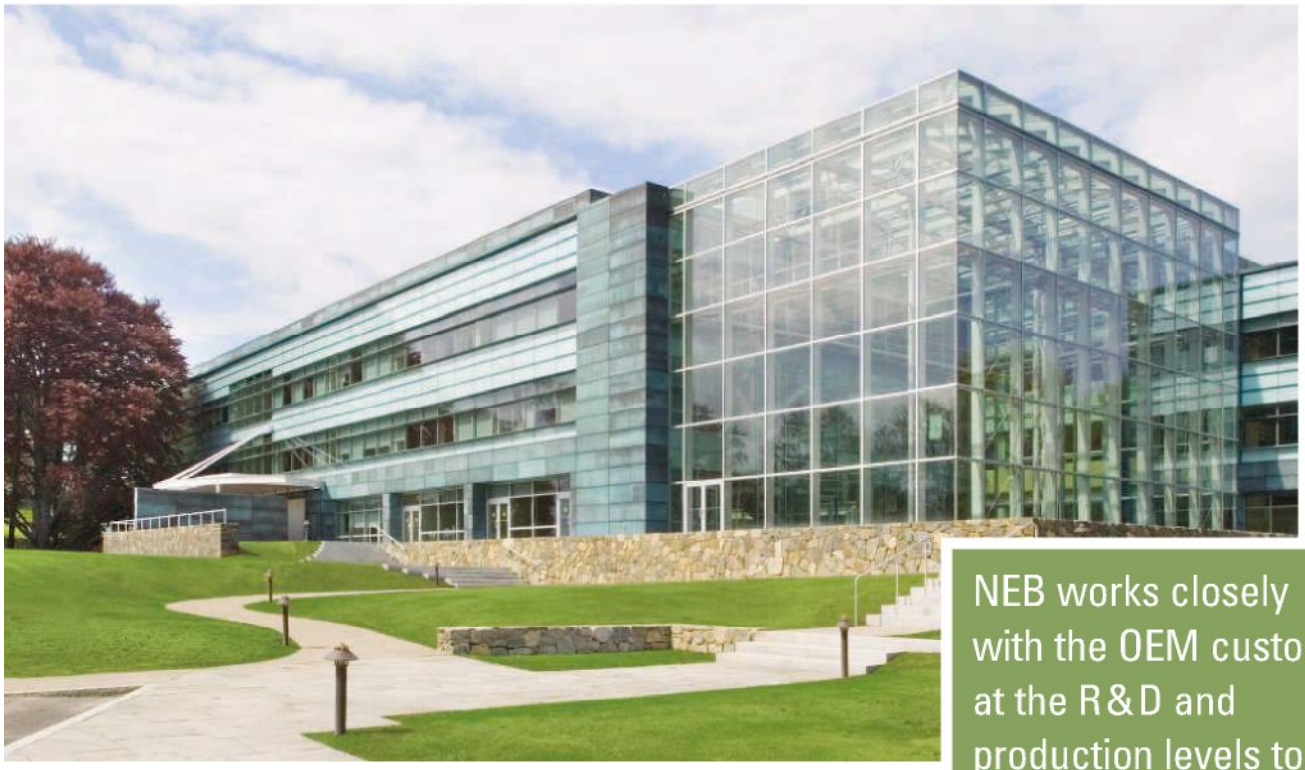
The U.S. Army Corps of Engineers built most of the New Orleans flood-control system in the 1960s, including levees to withstand a Category 3 storm. Katrina blew in as a Category 4, packing winds up to 217 kilometers per hour. But although the storm surge on the city's east side, closer to the hurricane's eye, sent water over the tops of the levees, downtown areas to the west faced winds and surges consistent with a Category 1 hurricane, with a recorded peak of 139 km/h. Two teams of independent civil engineers presented field evidence that the western levees gave way before water reached their tops, supporting early speculation along those lines. "This was a preventable disaster," said Ivor van Heerden, a hurricane expert at Louisiana State University (LSU) in Baton Rouge who is investigating the disaster for the state.

The engineers told legislators that they couldn't determine whether the failures occurred because of poor design or bad construction. But their data are sure to play a role in any political recriminations and the expected surge of civil suits. "Many of the widespread failures throughout the levee system were not solely the result of Mother Nature," said Senator Susan Collins (R–ME), chair of the Senate committee on Homeland Security and Governmental Affairs, which conducted the 2 November hearing. "Rather, they were the result, it appears, of human error in the form of design and construction flaws." The corps says that it's too early to draw any conclusions.

LSU scientists issued forecasts on the night before the hurricane struck that New Orleans would flood (*Science*, 9 September, p. 1656). But they assumed that the levees would not fail and predicted water only in the eastern areas. Van Heerden, using computer models, and the engineers, ▶

Through the breach. Engineers say the 142-meter gap in the 17th Street levee was caused by a damaged foundation. It took 5 days to close.

CREDITS (TOP TO BOTTOM): AVENTIS PASTEUR; U.S. ARMY CORPS OF ENGINEERS



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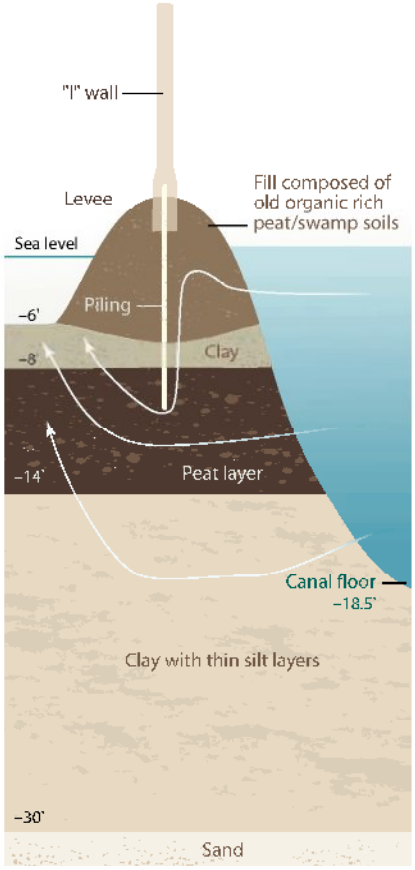
citing observations, concluded that water reached only 3.7 m up the 4.3-m levee walls lining the 17th Street and London Avenue canals. Independent modelers, led by civil engineer Joannes Westerink of the University of Notre Dame in Indiana, give similar initial results. "The water should be able to be filled chock-a-block to the top of the wall," said coastal engineer and team member Tony Dalrymple. "[The levees] didn't fill, [but] they failed anyway."

The 17th Street Canal burst through its banks at about 10:30 Monday morning, possibly after water penetrated, eroded, or lubricated the soil below the walls. "It's kind of like a layer cake, and the whole thing slid," says civil engineer Thomas Zimmie of Rensselaer Polytechnic Institute in Troy, New York, a member of the NSF effort. The levee became a bulldozer as the embankment slid 14 meters laterally, lifting and shoving trees, a shed, and a fence as water rushed in all around.

Evidence found at the London Avenue Canal, which breached at about 9:30 a.m., suggested that sand deep below the concrete levee wall had become saturated and unstable, causing the levee to tip. Soil movement, the corps acknowledged in a prepared statement, "could have been a factor" in the breaches. Those failures led to flooding in areas including Lakeview, Gentilly, and downtown. Other breaches, caused by overtopping, led to more inundation.

A fundamental factor in the strength of a levee—especially in swampy soil—is the depth of metal sheet piling driven deep below the levee as an anchor. Documents suggested that the sheet piling at the London Avenue Canal went about 5 meters down—half the depth found in other areas. But engineers said they lacked definitive data.

Those testifying proposed several low-cost improvements including filling gaps between levee sections, more consistent construction standards, and a national board to inspect levees. Van Heerden also called for strengthening the levees to withstand a Category 5 storm, a more expensive fix. A joint report on the levee system by the corps and other federal agencies is due out in July 2006. —ELI KINTISCH



Washout. An investigative team with the state of Louisiana has proposed three ways in which the 17th Street levee in New Orleans was undermined and breached during Hurricane Katrina.

CREDIT: (TOP TO BOTTOM) ADAPTED FROM DIAGRAM BY I. VAN HEERDEN, UNIVERSITY OF WISCONSIN, MADISON

ITER

Fusion Leaders Make a Diplomatic Choice

CAMBRIDGE, U.K.—A Japanese diplomat has been chosen to head the International Thermonuclear Experimental Reactor (ITER) project, the world's most expensive scientific collaboration. Meeting in Vienna this week, representatives of the six international partners in the project—China, the European Union (E.U.), Japan, Korea, Russia, and the United States—tapped Kaname Ikeda to lead the $12 billion fusion project, which aims to build a reactor to recreate the sun's power source.

Ikeda, currently Japan's ambassador to Croatia, has a degree in nuclear engineering and has held numerous positions in the Atomic Energy Bureau of Japan's Science and Technology Agency, the Ministry of International Trade and Industry, and the National Space Development Agency. "He has wide experience and seems to be an excellent choice," says Chris Llewellyn Smith, head of U.K. fusion research.

Since choosing a site earlier this year (*Science*, 1 July, p. 28), ITER negotiators have been drawing up an international agreement. Although this delicate process may continue well into next year, an E.U. source says that construction could begin at Cadarache in France as soon as a few weeks from now.

However, delegates in Vienna failed to agree on the inclusion of India as a partner in the project. India had asked to join in July, but sources say that some ITER partners do not want India to have a prominent role because of its failure to sign the Nuclear Non-Proliferation Treaty. —DANIEL CLERY

ScienceScope

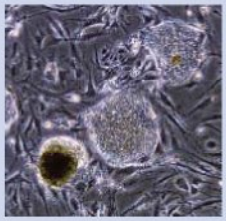
Hot on the Toxin Trail

David Schwartz, director of the National Institute of Environmental Health Sciences, has previewed a proposed $4 million program that will spur the development of new technologies to detect, measure, and track toxins both in people and in the environment. If all goes as planned, the Exposure Biology Initiative will develop sensor badges or bracelets to give researchers more precise data linking toxins to health. The plan also calls for techniques that will monitor protein-toxin interactions that may serve as early markers of problems, he reported at last week's Environmental Epigenomics Conference in Durham, North Carolina. Schwartz is setting up a meeting this winter to home in on specific goals, and he hopes to get the initiative up and running in 2006. —ELIZABETH PENNISI

The Endless Battle Over Stem Cells

Advocates for human embryonic stem (hES) cell research are applauding a veto last week by Wisconsin Governor Jim Doyle of a bill that would have banned all forms of human nuclear transfer research. But it's no time to relax, says Sean Tipton of the Coalition for the Advancement of Medical Research, an hES cell research lobby group: The issue is heating up in at least three more states.

In Florida, groups are collecting signatures for competing amendments to the state constitution. One would make available $200 million in state grants for research on hES cells; the other would ban state funding for work that "involves the destruction of a living human embryo." Both initiatives must collect 600,000 signatures and be approved by the state Supreme Court to make it onto the November 2006 ballot. In Missouri, where several legislative attempts to limit hES cell research have been defeated, former U.S. Senator John Danforth (R–MO) is heading a committee to collect 150,000 signatures to put a constitutional amendment on next fall's ballot that would specifically allow hES cell and nuclear transfer research. In Ohio, which in 2003 became one of the first states to fund hES cell work with state money, several bills are pending that would limit or even ban such research. "I imagine it will be a busy winter," Tipton says. —GRETCHEN VOGEL

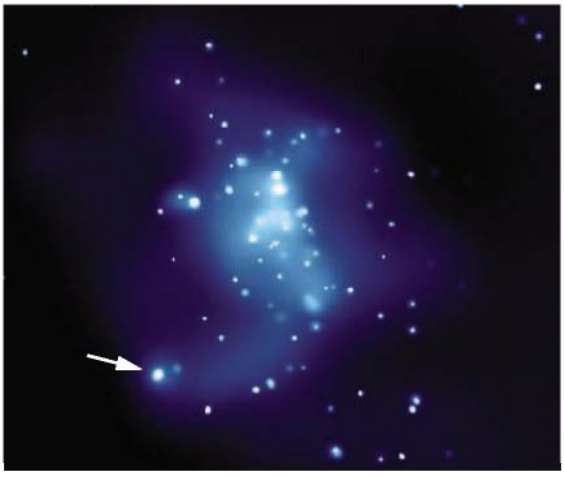


ASTROPHYSICS

Surprise Neutron Star Suggests Black Holes Are Hard to Make

Black holes may be harder to create than previously believed, according to an unexpected discovery made with NASA's Chandra X-ray Observatory. Researchers have long thought that any star more than 25 times the mass of our sun will end its life as a black hole. But Chandra's finding suggests that even a star of 40 solar masses may fail to create one. Because such massive stars are extremely rare, that raises the question of how stellar black holes form at all. "It's a surprising find," says Gertjan Savonije of the University of Amsterdam, the Netherlands.

When a massive star exhausts its nuclear fuel and goes supernova, it blasts its outer mantle into space. The remaining core collapses into a small, dense neutron star or a black hole: a region of space where gravity is so strong that not even light can escape it. Astrophysicists used theories of stellar evolution to peg 25 solar masses as the threshold above which anything will end up as a black hole. "There's a lot of guesswork involved," says Savonije, because stars blow off varying amounts of gas into space during their lifetimes. Even so, the figure gave astronomers some idea of what to expect when viewing stellar corpses.



Confounding fate. The stellar giant that produced this neutron star should have ended as a black hole, researchers say.

So a team led by Michael Muno of the University of California, Los Angeles, was surprised to find the pulsating x-ray emission of a neutron star in a young, massive, compact star cluster known as Westerlund 1. In such clusters, the stars are thought to have been born all at the same time, in this case, about 4 million years ago. More-massive stars have shorter lives because they burn more fiercely, so the progenitor of the neutron star (designated CXO J164710.2–455216) must have been one of the most massive stars in the cluster. As there are 35-solar-mass stars still around in the cluster, the researchers guess the neutron star progenitor must have been at least 40 solar masses. In a paper accepted for publication in *Astrophysical Journal Letters* (arxiv.org/abs/astro-ph/0509408), Muno and colleagues conclude that some of the most massive stars do not become black holes as predicted.

Why not? Frank Verbunt of Utrecht ▶

NATIONAL SCIENCE FOUNDATION

Board Suggests How to Thrive Under Stress

When money's tight, it's important to let the experts call the shots—but make sure they aren't too conservative. That advice comes from the governing body of the U.S. National Science Foundation, which has drafted a long-term plan for running NSF without the promised doubling of its budget. The National Science Board's (NSB's) prescription: Give project managers more leeway, and don't let grants to large centers erode support to individuals.

Coincidentally, the board issued its plan 1 day before a key legislative spending panel approved a surprisingly generous 2006 budget for NSF. It's generous only in comparison to the president's requested 2.4% boost and earlier congressional action, however; The 3% increase will barely keep the agency ahead of inflation.

Senator Kit Bond (R–MO) originally requested the report as chair of NSF's appropriations panel. Although his panel no longer has jurisdiction over the agency, NSB chair Warren Washington said the board wanted to reexamine NSF's policies anyway after concluding that current economic conditions had destroyed hopes of a 5-year doubling of NSF's budget spelled out in a 2002 reauthorization. "It's still a big disappointment to me that it hasn't happened," Washington says, noting that NSF's budget would be about 50% larger by now if the doubling had begun on schedule. "But in the meantime, we wanted to emphasize what NSF needs to do to keep the country's basic science enterprise strong."

One essential step, according to the draft plan, "2020 Vision for NSF" (www.nsf.gov/nsb; NSB 05-142), would be to strengthen the hand of program officers in choosing from among a surfeit of good research proposals. It's part of the board's hunger for more "transformative" research (*Science*, 8 October 2004, p. 220): experiments with the potential to radically change a field rather than simply add incrementally to what is known. "Often a program officer will get a mixed set of reviews," says Washington. "We shouldn't allow one negative review to kill a proposal if the program officer thinks that it's worth taking the risk."

The board also weighed in on the never-ending debate about the proper balance between the number, size, and duration of grants. It suggested that NSF should fund a larger and more diverse pool of researchers, even if it means suboptimal funding of individual grants and fewer large awards. And it challenged NSF administrators to "increase the impact" of its science education programs, a portfolio that the Bush Administration has tried to cut sharply in the past 2 years, by doing a better job of applying new research findings to the classroom.

The board's ideas are "perfectly consistent with our initiatives," says NSF Director Arden Bement. Program officers already have the ability to seed novel ideas, he notes. But Bement says he'd also welcome congressional authority to add staff, to relieve the growing workload and allow program officers to be even more creative.

Last week's budget action, by House and Senate conferees, would give NSF an additional $164 million, for a total of $5.64 billion. NSF's research account would grow by $155 million, to $4.38 billion, and its education programs would drop by only $36 million, to $805 million, rather than by the $104 million cut requested by the Administration. The bill would fund all new research facilities requested in 2006 except for the high-energy RSVP project at Brookhaven National Laboratory in Upton, New York.

–JEFFREY MERVIS

CREDITS (TOP TO BOTTOM): NASA/CXC/UCLA/M. MUNO ET AL.; NSF

University in the Netherlands says that if the progenitor star had been part of a binary system, its companion could have siphoned off enough mass to keep the giant star from collapsing into a black hole. "Recent evolutionary calculations show that in a binary scenario, you almost always end up with a neutron star," Verbunt says.

Muno agrees that such a scenario is possible. But if the neutron star is still sitting in the cluster, its bloated binary partner should still be around too—yet, Muno says, infrared observations of the neutron star reveal no binary companions more massive than our sun. "It's still important to consider other reasons why some extremely massive stars won't collapse into black holes," he says.

–GOVERT SCHILLING

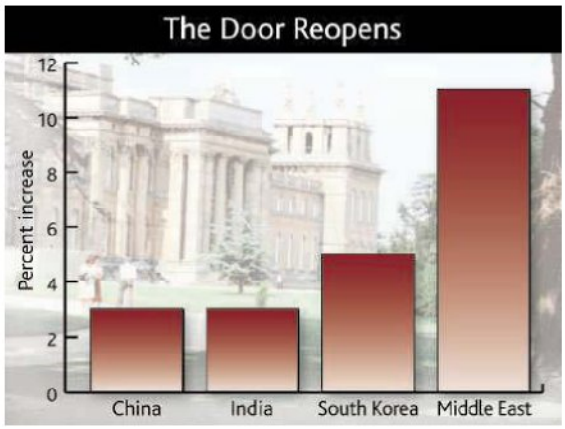
Govert Schilling is an astronomy writer in Amersfoort, the Netherlands.

U.S. HIGHER EDUCATION

Schools Cheer Rise in Foreign Students

The number of foreign students enrolling in U.S. graduate programs has gone up this fall for the first time since 2001. Educators attribute the 1% increase over last year, documented in a survey by the Council of Graduate Schools (CGS), to improvements in visa processing and see it as the reversal of a trend that began after the 2001 terrorist attacks. But they remain concerned that the United States may still be losing its attractiveness as a destination for students from around the world.

The increase, reported by 125 institutions that responded to a survey of 450 schools, comes in spite of a 5% decline in international applications compared to last year. The number of students from China and India—the two largest sending countries—has risen by 3%, and enrollments from the Middle East are up by 11%. Engineering enrollments, a top draw among international students, are up 3%, and the physical sciences recorded a 1% rise.



Renewed welcome. Faster U.S. visa processing has boosted graduate enrollments from the biggest pools of international talent and the Middle East.

"Visa problems—the main factor that hobbled enrollments in recent years—are clearly being addressed," says CGS president Debra Stewart, who on a recent visit to the U.S. consulate in Beijing learned that approvals of student visa applications had shot up to more than 80% from less than 50% a year ago. "Things are moving in a good direction."

But Heath Brown, CGS's director of research and policy analysis and the author of the study, finds it troubling that a smaller fraction of international students—38% compared to 43%—chose to enroll after being accepted. "It's possible that some of these students are going to other countries," says Brown. Stewart says one way for the United States to stay ahead in the global competition for talent would be to make it easier for foreign students with advanced U.S. degrees to gain permanent residency.

John Martin of the Federation for American Immigration Reform in Washington, D.C., thinks that would be a bad idea. "We think the increased enrollment of foreign graduate students, particularly in science and math, has discouraged American students from pursuing scientific and engineering careers," he says. "Foreign graduate students who end up with science and engineering jobs in this country tend to hold down salary increases in those fields, so it's natural for American students to pursue fields like law in which they see greater economic rewards." As a consequence, Martin says, the United States is becoming more and more dependent on foreign scientific talent.

Unlike Martin, most university officials are hoping this year's increase will turn out to be the beginning of an upward trend. Sherif Barsoum of the Office of International Education at Ohio State University in Columbus is particularly encouraged by the numbers from the Middle East. "The post–9/11 perception of U.S. campuses being unfriendly to foreign students seems to be fading," he says.

–YUDHIJIT BHATTACHARJEE

SOURCE: COUNCIL OF GRADUATE SCHOOLS; P. KOTOK.COM

ScienceScope

Europe to Cut Lab Animal Tests

European governments and industry plan to reduce animal testing and develop better alternatives. On 7 November, the European Commission (EC) and leading industry associations agreed to cut the number of animals used for basic research, toxicology, and quality control of health products from 11 million a year to 9 million by 2007.

Although lean on specifics, the agreement should also help coordinate research activities to develop animal-friendly methods, such as cell cultures and computer modeling. The parties will work together to facilitate the official validation of methods and ease the regulatory acceptance process. "We have never before had the opportunity to work together in such an integrated manner," says Alain Perroy of the European Chemical Industry Council.

The EC has promised to add an unspecified amount to the $16 million it already spends each year on alternative testing methods.

–XAVIER BOSCH

U.S. Science Budgets Emerge

A month into the new fiscal year, the 2006 budget is finally taking shape, and U.S. science lobbyists are cautiously optimistic. Under a consensus bill passed by a joint House-Senate committee this week, the Department of Energy's Office of Science would receive $3.63 billion, a 1% rise over 2005 and $170 million more than the White House requested in February. Funding for nuclear bunker-buster research sought by the Pentagon was not granted, and the National Ignition Facility superlaser at Lawrence Livermore National Laboratory in California escaped a Senate attempt to close it.

Lobbyists also cheered continuation of the National Institute of Standards and Technology's Advanced Technology Program, seen as corporate welfare by congressional critics, and a 9% boost to the president's request for the National Oceanic and Atmospheric Administration, to $3.9 billion. NASA will get $16.5 billion, the requested amount, and $260 million more than last year, although Administrator Michael Griffin told lawmakers last week that a shuttle shortfall of up to $5 billion could eat into applied research.

One last concern is a feared 11th hour across-the-board rescission to make room for disaster relief and the Iraq war. "I've seen people saying everybody has to take their medicine," says Robert Boege of the Alliance for Science & Technology Research in America. –ELI KINTISCH

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The annual $25,000 Eppendorf and *Science* Prize honors young scientists for outstanding contributions to neurobiology research. Dr. Xu is the fourth recipient of this prestigious award, and he will be honored at a ceremony held during the week of the 2005 Annual Meeting of the Society for Neuroscience.

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EPIDEMIOLOGY

Russian Cancer Study Adds to the Indictment of Low-Dose Radiation

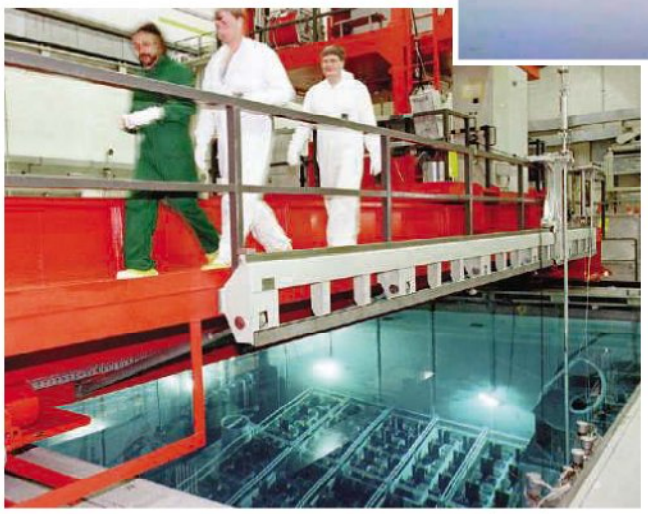
A Cold War environmental calamity appears to be the cause of a spate of cancers in the Russian heartland. A landmark study this month by U.S. and Russian scientists blames excess cancers in the Ural Mountains on chronic exposures to radioactivity leaked from a weapons plant a half-century ago.

The study is the latest blow to the notion that there is a threshold of exposure to radiation below which there is no health threat (and there might even be a benefit). The results add weight to last summer's report from the U.S. National Research Council, which backed the hypothesis that radiation is risky even at the smallest doses (*Science*, 8 July, p. 233). Although that conclusion had been inferred from Japanese atomic bomb survivors, the Russian study—along with a recent report revealing an elevated cancer risk in nuclear workers around the globe—provides the strongest direct evidence yet of chronic, low-dose health effects.

Both sets of findings indicate that workplace radiation standards are correct in erring on the safe side. In 1991, the International Commission on Radiological Protection (ICRP) set an annual workplace limit of 20 millisieverts (mSv) per year over 5 years, which assumes there is no safe level. "This is an endorsement of the precautionary approach as a tool for radiation protection," says Lars-Erik Holm, director general of the Swedish Radiation Protection Authority and ICRP chair.

The new data come from villagers downstream from the Mayak weapons complex in the southern Urals, victims of the struggle for nuclear supremacy. From 1949 to 1956, they were exposed to a steady stream of plutonium production byproducts released into the Techa River. After the Soviet breakup, U.S. experts—including atomic bomb radiation expert Dale Preston, now at Hirosoft International Corp. in Eureka, California, and epidemiologist Elaine Ron of the U.S. National Cancer Institute—joined forces with colleagues at the Urals Research Center for Radiation Medicine in Chelyabinsk to scrutinize the health of 25,000 people who lived in 41 villages along the Techa between 1950 and 1952, when radioactivity releases climaxed, and nearly 5000 people who moved to these communities between 1953 and 1960.

The biggest challenge has been getting a handle on individual radiation doses, which remain uncertain. The team has measured strontium-90, the most common downstream radioisotope, in teeth from scores of subjects and conducted whole-body counts of strontium and cesium-137. They have at least one strontium measurement for more than a third of the villagers.



Low-dose risks. A study of cancers in Muslyumovo (*inset*) and other villages near the radionuclide-laden Techa River points up the importance of limiting exposure to radiation in the workplace.

According to death certificates, 1842 villagers died from solid tumors other than bone cancer, the prevalence of which would have been skewed by strontium-90. And 49 died from leukemia, not counting chronic lymphocytic leukemia (CLL), which is not thought to be triggered by radiation. The researchers attributed deaths above the background rate to radiation—46 from solid cancer (2.5%) and 31 from leukemia (63%). The risks increase with estimated dose, the team reports in the November issue of *Radiation Research*. "People were hoping that the risks would be a lot lower," says Lynn Anspaugh, now at the University of Utah, Salt Lake City, who helped with the study's dosimetry.

The figures, although alarming, are in line with the largest study of nuclear power workers ever carried out. A team led by Elisabeth Cardis of the International Agency for Research on Cancer in Lyon, France, pooled data on more than 400,000 plant workers in 15 countries. In this group, 6519 have died from solid cancers and 196 from non-CLL leukemias. The finding suggests that between 1% and 2% of the deaths may be due to radiation, the team concluded in the 29 June issue of the *British Medical Journal*.

It's an "impressive study," says Holm, although he and others flagged a shortcoming: Smoking may account for a large share of deaths attributed to radiation. In the study, the risk of smoking-related tumors—primarily lung cancers—is much higher than for other solid cancers. Cardis points out that the paper acknowledges smoking as a confounding factor. "Although smoking may play a role in the increased risk of all cancers excluding leukemia, it is unlikely to explain all of the increased risk observed," she says. Future publications will address concerns about the study's methods, she says.

Although the Cardis study has been challenged, exhibit B in the low-dose indictment, the Techa River study, provides corroborating evidence. The two studies come to "practically the same conclusion," says Peter Jacob of the Institute of Radiation Protection in Neuherberg, Germany. That means that the 20-mSv standard is unlikely to budge, despite arguments from industry that it is too stringent. The Russian results are "a setback for those who hope for a relaxation of the standards," says Anspaugh. The United States is one of the few nations that does not use the ICRP standard; it permits exposures up to 50 mSv per year.

In practice, most nuclear industry workers are exposed to far less radiation than the ICRP limit. That's a good thing: The average lifetime dose in the Cardis power plant study was only 19.4 mSv, with less than 0.1% of workers receiving more than 500 mSv. Calculations in the Techa study suggest that the vast majority of villagers received less than 50 mSv of lifetime accumulated dose to the stomach. In light of ongoing efforts to refine these estimates, says Urals center director Alexander Akleyev, cancer risks should be viewed as "preliminary." Jacob agrees: "This is not the final word," he says. –RICHARD STONE

Researchers have dug up some surprising evidence casting doubt on the long-held belief that microbes are impervious to geographic constraints

Biogeography: Is Everything Everywhere?

How would the world look if marsupials, instead of being confined to the sanctuary and prison of Australia, had been forced to confront every other carnivore, tree-climber, burrower, and grazer on the planet? Our ideas about ecology, evolution, and history would be quite different had they been derived from studying animals that had crossed oceans, mountains, or deserts to seek out suitable environments. Polar bears in the Antarctic? Penguins in Alaska? Chimpanzees in Amazonia? Kangaroos on the Serengeti?

The picture seems far-fetched. Yet for about a century, microbiologists have believed that the organisms they study are unhindered by geographic boundaries, traveling the world and thriving wherever they find their preferred environment—be it hot springs, freshwater ponds, or rotting fir trees. That view gives researchers who study microbes a rather different perspective on the world. As the Dutch biologist Lourens Bass-Becking put it in 1934: "Everything is everywhere; the environment selects."

Or maybe not. In the past few years, many microbial ecologists have come to believe that microbes are not infinitely mobile. Bass-Becking's dictum is really only "an assumption," says Jessica Green of the University of California, Merced. "It's based on a confusion of hypotheses for facts."

DNA studies have given us a more detailed picture of microbial diversity that, argue some, demands a more nuanced view of microbial ecology. Those nuances have spawned a debate over what the DNA data actually show, and how a molecular view of microbial diversity can be compared with our species-based view of plant and animal ecology. Answering those questions, in turn, will help scientists better understand the crucial role played by microbes in keeping our ecosystem livable.

On Priest Pot

Bland Finlay of the Centre for Ecology and Hydrology (CEH) in Dorset, U.K., has spent a quarter of a century building up evidence to support Bass-Becking's view of microbial ubiquity, much of it gathered in a small lake in northern England called Priest Pot. For example, he has found that a mere 25 microliters of sediment from Priest Pot contains 40 of the world's 50 known species of the protozoan *Paraphysomonas*. What's more, each species' abundance in the sample matches its abundance worldwide. Everywhere he goes, Finlay finds identical ciliates: "There's no convincing evidence for endemic species," he says. "I see the same ones in Scotland, New Zealand, and central Africa."

The main cause of microbes' ubiquity is their vast populations, says Finlay. Although a specific ciliate is extremely unlikely to make a long journey, there are so many of them that some inevitably will hitch a ride via wind, water, a bird's foot, or a clump of floating vegetation. Many can tolerate a wide range of environments—salt- and freshwater, for example—and they have an astonishing ability to hunker down in harsh environments until their moment arises.

Cultured in its native conditions, a gram of Priest Pot sediment yields 20 species of ciliate protozoan. But when Finlay's team tested that sediment in the lab under different conditions—altering salinity, temperature, illumination and so on—it found 137 species. And the total keeps rising. He thinks those findings argue strongly for the idea that the lake contains not only all the species adapted to its conditions but also a "seed bank" of many others that have arrived and survived, but not thrived. Everything seems to be everywhere, even if it is not immediately obvious. "There is no biogeography for anything smaller than 1 millimeter," he says.

Lake effect. Bland Finlay has plumbed Cumbria's Priest Pot for a quarter-century of discoveries involving ciliate protozoa.

CREDITS: (TOP) T. J. BEVERIDGE/VISUALS UNLIMITED; (BOTTOM GROUP) B. J. FINLAY/NERC

But Green believes that our understanding of microbial diversity is too sketchy to support such statements. "[Finlay] has shown that there's similarity at certain points, but the sampling effort on a global scale isn't enough to make these sweeping generalizations," she says. Green is one of a wave of researchers using molecular studies to probe the patterns in microbial diversity. The ability to sequence DNA samples from the environment has allowed scientists to detect far more than the 1% of microbes that can be cultured in the laboratory. It has also revealed how they vary from place to place.

Studying ascomycete soil fungi in the Australian desert, Green and her colleagues have found that the genetic differences between fungi from different locations increase with distance. Others have found that archaea of the genus *Sulfolobus* living in the hot springs of Yellowstone and Lassen U.S. national parks, for example, are more similar to each other than to those found on Russia's Kamchatka peninsula. "We are beginning to see biogeographic patterns in microorganisms," says Claire Horner-Devine of the University of Washington, Seattle, lead author of a study of New England salt-marsh bacteria with similar results. "There will be organisms that are global and can get anywhere, and you'll also find ones that don't have those ranges."

Digging in. Jessica Green pursues *Nitrosomonas* bacteria at two high-altitude locations in Chile.

Biologists studying plants and animals realized 150 years ago that the number of species found in a patch of habitat climbs as the area of the patch increases, a biogeographic pattern called the species-area relationship. A CEH team led by Christopher van der Gast recently argued that the same held for the bacteria living in oil-filled sump tanks in engineering machines and in water-filled tree holes in Amazonia. This seems to contradict the "everything-is-everywhere" view, in which the relationship between a place's area and the microbial species it contains is essentially flat.

One complication is that limited dispersal is not the only thing that could create geographic variation in microbes. A big challenge is to separate the effect of environmental heterogeneity—which everyone accepts will cause biological differences—from divergences caused by dispersal. Finlay and his colleague Tom Fenchel of the University of Copenhagen, Denmark, have argued that van der Gast's tree-hole study found more diversity in larger sites because larger sites are environmentally more heterogeneous, not because they are easier to disperse into or harder for populations to go extinct in.

"The next frontier is to figure out whether the patterns are due to environmental selection or to evolution and diversification," says Jennifer Hughes of Brown University. She says a handful of published studies so far show geographical patterns when environmental differences are controlled for.

Phenotype matters

More vexing is the issue of how to make sense of the molecular data themselves. Some believe that microbes seem ubiquitous because our view of them is blurry. Many studies assign microbes to different species if their ribosomal DNA is less than 97% identical. If that were done with animals, Green points out, all primates from humans to lemurs would likely be lumped into one category—creating a group with far more cosmopolitan distribution and habits than any of the species erected by traditional naturalists. What's needed, she says, is a study that would detect whether and how the patterns in microbial diversity compare with those seen in plants and animals—at scales from a cubic centimeter to intercontinental. She aims to do this for the bacteria in Mediterranean-type ecosystems in Chile, California, and South Africa.

But Fenchel believes that simply comparing DNA seq-uences misses biological reality. Microbial species tend to be very old, he says, and have accumulated a lot of "neutral" genetic variation that has no evolutionary effect. If you look hard enough, he argues, every individual will be different.

Fenchel favors classifying microbes by what they look like and what they do. "The molecular data are super, but you shouldn't forget the phenotype—and some of the molecular chaps do," says Fenchel. "A couple of years ago, people thought genetic analysis was the bees' knees and that it would clear up all the questions," adds Finlay. "I don't think this is true at all." And many microbiologists believe that the ability of distantly related bacteria to swap DNA may further confuse our picture of their diversity and distribution.

The debate about microbial biogeography is about more than how many bacteria can dance on the head of a pin. Microbes support the visible living world and provide trillions of dollars' worth of ecosystem services for free, cleaning air and water and keeping soil fertile and healthy. They are a critical component of efforts to restore degraded ecosystems. As pathogens, they help regulate the populations of plants and animals, and their absence may be one factor behind the success of invasive species.

To understand these processes, says Horner-Devine, we must understand microbes' ecology and how they will respond to stresses such as climate change and pollution. "To know how we're affecting these communities, we need to know what the patterns in spatial and temporal variations are," she says. Such knowledge will help build a biology that applies to all life on Earth.

"Comparing microorganisms with plants and animals will highlight where we see patterns and processes that could be the same for all domains of life," says Horner-Devine. "That would be pretty phenomenal."

—JOHN WHITFIELD

John Whitfield, a science writer based in London, is the author of the forthcoming book *In the Beat of a Heart: The Search for a Unity of Nature*.

CREDITS (TOP TO BOTTOM): (INSET) DENNIS KUNKEL MICROSCOPY, INC.; P. TRAVA...; M. JENNIFER DUNNE

The Baroness and the Brain

Best known for her popular writing, neuroscientist Susan Greenfield has launched a new center at Oxford to investigate consciousness

OXFORD, U.K.—Chatting amicably around a long oval table sit a couple of dozen researchers interested in how the brain works. This is the first gathering of the Oxford Centre for the Science of Mind, an ambitious project involving people with a diverse set of skills and interests. Today's first order of business is to choose a keynote speaker for a conference on consciousness next year. All eyes turn to a commandingly tall woman with leonine features, Director Susan Greenfield, as she throws out a suggestion: "How about the Dalai Lama?" There are chuckles around the room, but it soon becomes clear that Greenfield is serious—and that she could probably make it happen.

A neuroscientist at Oxford University for 30 years, a politician, and celebrity, Greenfield rose through the academic ranks like a bottle rocket, but she didn't stop there. Over the past decade, she has become a household name in the United Kingdom, the author of 10 popular science books, the host of a TV series about the brain, and the first woman director of London's Royal Institution, a 200-year-old venue for the public understanding of science. Along the way, she has been tapped as a scientific adviser by both the U.K. and Australian governments. In 2001, she became a lifetime member of the U.K. House of Lords with real decision-making power.

"She has been immensely energetic and effective," says Martin Rees, an astrophysicist and Master of Trinity College at the University of Cambridge, U.K., "expounding and debating scientific ideas and issues to a wider range of audiences than most scientists ever reach." But critics say Greenfield's ascendancy has been fueled by self-promotion rather than published research. They grumble that she appears to have left real science behind without delivering on the promise of her early ideas.

Science rock star

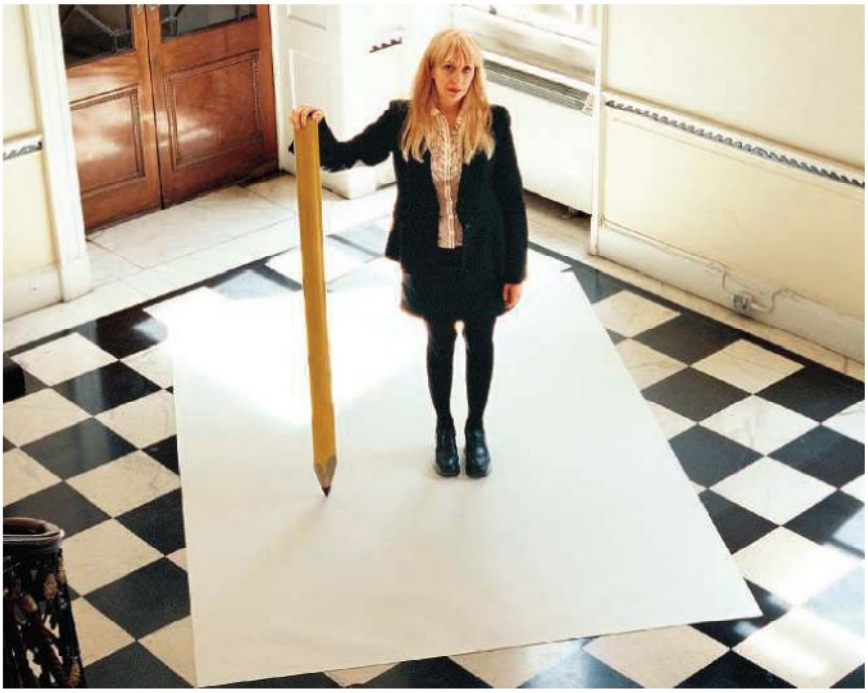
When it comes to the media, most scientists are shy creatures, preferring the snail's pace of peer-reviewed journals to the glamour—or terror—of a 30-second TV interview. Not Greenfield. She comes alive in the spotlight. "I get a terrific kick out of engaging with the public," she says. "As an academic, you just sit there with all the ideas and have very little influence, … but I'd rather see my ideas translate to policy that makes a difference in people's lives."

Greenfield's early career gave no clue that the neuroscientist, now 55, would become "the 14th most powerful woman in the U.K." and one of "the 300 most influential people in the world," as two British newspapers have ranked her. Her research has centered on a workhorse molecule of the nervous system called acetylcholinesterase (AChE).

"She first made a name for herself with a very bold idea about AChE," says Hermona Soreq, a neuroscientist at the Hebrew University in Jerusalem, Israel; namely, that the enzyme might be a link between several neurodegenerative diseases—Alzheimer's, Parkinson's, and possibly also motoneuron disease—"but not as an enzyme." Whereas an enzyme's job is to catalyze a chemical reaction—AChE splits the neurotransmitter acetylcholine into choline and acetic acid—Greenfield proposes that AChE does more: It may also interact with proteins to stimulate neuron growth during development, and this pathway may become deranged in the adult brain, she believes, leading to neuronal death and other symptoms shared by neurodegenerative diseases. "If her idea turns out to be true, it would be an amazing breakthrough," says Jean Massoulie, a neuroscientist at the National Center for Scientific Research in Paris, France. But, he adds, "in my view, it is still not proven that AChE even has nonenzymatic roles."

Everything changed for Greenfield in 1994 when she was invited to give the annual Royal Institution (RI) Christmas lecture on television, the first woman to do so. Soon after that lively presentation on brain function, she says, "one thing just led to another." She began writing regular columns for newspapers, weighing in on hot topics such as whether marijuana should be legalized—Greenfield believes not—and producing popular books about the brain. Greenfield became a familiar face on television. She even appeared in the U.K. tabloid magazine *Hello!*

In 1998, Greenfield was tapped to be director of the RI—again, the first woman so honored—running Britain's oldest institute for showcasing science. In 2001, a committee of U.K. politicians appointed her a member for life of the House of Lords as part of an effort to include nonpolitical experts in the legislative branch. Now known as the Baroness of Otmoor in the County of Oxfordshire, Greenfield can vote on laws, although she says her "most important contribution there is to take part in debates."



Big agenda. Dubbed Britain's "14th most powerful woman" by the press, Susan Greenfield is a skilled attention getter—here in an appeal for new high-tech ventures.

CREDIT: PHOTO BY ALAN BURLES, AS PUBLISHED ON WWW.SPACEFORIDEAS.U.K.COM

But Greenfield is interested in more than talk; she wants to put ideas into action. One of her initiatives, called the Science Media Center, offers briefings for journalists on scientific issues and rallies researchers willing to be interviewed on short notice. "It makes a tremendous difference," says David King, a chemist at the University of Cambridge and the U.K. government's chief science adviser, particularly with fast-breaking news, such as the current threat of an avian influenza pandemic, in which disinformation can cause panic.

Greenfield is now working on a plan to establish a Science Peace Corps in the United Kingdom modeled on the U.S. Peace Corps. Scientists would spend a year or two in the developing world, broadening their horizons while sharing their expertise.

Meanwhile, Greenfield, who is single, says she still maintains a research laboratory at Oxford, when she isn't flying around the world to collect honorary degrees—28 so far—or achievement awards. Her day begins at 5 a.m., but still, she says, "life is too short."

At odds with her peers

Widely admired by the public, Greenfield nevertheless gets mixed reviews from her scientific peers. Although she has become one of the United Kingdom's high profile "science ambassadors," says King, she has taken an unusual path. "A good comparison," he says, "is Lord [Robert] May," an Oxford biologist who was also appointed to the House of Lords in 2001. "Everyone considers him to be one of the most important epidemiologists in the world, but when people are asked about Susan's background, they falter."

Greenfield's new venture into the field of consciousness research is raising more hackles. Her Oxford Centre for the Science of Mind (OXCSOM) has received $2 million in start-up funding from the U.S.-based Templeton Foundation (*Science*, 21 May 1999, p. 1257), and she could receive a further $10 million next year. Greenfield admits she has never done an experiment involving consciousness, although she has described her theory for how the activity of neurons creates individual minds in her popular books, which she describes as "the work I am most proud of."

In a nutshell, Greenfield argues that consciousness is generated by "highly transient assemblies of brain cells that wax and wane in size, from one moment to the next," and the larger the assemblies, the higher the level of consciousness. She uses the analogy of a stone dropped into a pond, with associations between neurons rippling out from a "trigger."

Greenfield gave a speech about her idea at the annual meeting of the Association for the Scientific Study of Consciousness, held at the California Institute of Technology (Caltech) in Pasadena in June. "It went extremely well," she told *Science* after the meeting, but some in her audience painted a different picture. Patrick Wilken, a psychologist at Otto von Guericke University in Magdeburg, Germany, and one of the

CREDIT: COURTESY OF S. GREENFIELD

conference organizers, says people complained that Greenfield's lecture was insubstantial—for example, some felt that "talks like this lower the perception of consciousness as a serious field of academic study." Christof Koch, a Caltech neuroscientist who chaired the meeting, calls Greenfield "an excellent public speaker" but says her talk had "very little science" and focused more on metaphors than testable hypotheses.

Greenfield calls the assessment unfair and claims she is being "held to a different standard" from others, perhaps "because I'm a neuroscientist and most of the others were cognitive scientists."

Wilken disagrees. A decade ago, "there were a number of researchers asserting [that they could] solve the problems of consciousness without having a great deal of data to back up their claims," he says, but "things have moved a long way since then, and people who make statements like this today without having let their ideas go through the normal scientific practice of peer review are generally ignored."

But Greenfield plans to get data to back her ideas with the help of OXCSOM. One of its research aims is "to test Susan's theory," says John Stein, an Oxford neurophysiologist and one of OXCSOM's core group of researchers, although "obviously we won't solve the problem of consciousness in a matter of months." In line with the religious interests of the Templeton Foundation, which bankrolls OXCSOM, its initial focus is on "the physical basis of beliefs."

For example, Oxford neuroscientist Irene Tracey is investigating whether religious beliefs affect pain tolerance. The pain is delivered to volunteers in the form of heat or a chili paste applied to the arm. Subjects who identify themselves as "deeply religious" use rituals to cope, such as praying, whereas nonreligious subjects just grit their teeth. Meanwhile, she uses functional magnetic resonance imaging to observe patterns of brain activity during the ordeal.

Capturing the brain's reaction is the easier part of the experiment, she explains, because it is readily detected. But to determine "how deep" beliefs are or "how much" pain is experienced, she must rely on reports from the subjects themselves. That subjective aspect is both a pro and a con. Although it can make comparisons very difficult without carefully chosen controls, it is also "exactly the aspect that we're trying to figure out," she says. "Pain is an incredibly flexible phenomenon, depending on your perceptions, expectations, and degree of self-awareness," all ingredients of consciousness. And on the practical end, determining the mechanisms that might dampen pain for a believer could lead to better therapies for everyone.

Academics sit and discuss ideas, but **"I'd rather see my ideas ... [make] a difference in people's lives."**

—Susan Greenfield

Whether grappling with slippery concepts such as belief will bring us closer to understanding consciousness is an open question. "But even if the project fails in its ultimate aim," says Erik Myin, a philosopher of consciousness at the University of Antwerp, the Netherlands, it could reveal how to convert such "big questions" into ones that can be scientifically validated.

But judging Greenfield on her own research may be missing the point. "She's gutsy and an inspiration" to younger scientists, says King. And among the public, "her ability to communicate that science is fun and creative" and that "you don't have to be a boring fuddyduddy wearing tweed skirts" is vital, says Stein. He says he can measure her impact every year in "the number of girls applying to do medicine or neuroscience who've said they've been enthused by Susan's lectures or books." Even if she doesn't crack consciousness, he says, Greenfield has already made an enormous contribution.

—JOHN BOHANNON

John Bohannon is a writer in Berlin, Germany.

Ancient DNA Yields Clues to the Puzzle of European Origins

DNA from prehistoric farmers adds fuel to a long-simmering debate over the ancestry of living Europeans; divergent male and female histories may help explain the contradictory data

In 2000, archaeologists uncovered a well-preserved male skeleton at an early farming site at Halberstadt, northwest of Leipzig, Germany. The skeleton was lying on its left side, its legs and arms tightly flexed, with three pottery bowls buried with it. The man had belonged to a central European culture called the Linearbandkeramik (LBK), characterized by large longhouses and distinctive pottery featuring sweeping striped designs. The LBK people, the first farmers known to occupy central Europe, arose in modern-day Hungary and Slovakia about 7500 years ago and within 500 years had spread as far west as France and as far east as the Ukraine.

Dead end? This prehistoric farmer from Germany had a DNA variant that is very rare today, suggesting that his farming techniques may have spread much farther than his genes.

For decades, researchers have studied the LBK culture for clues to how farming spread across Europe, an issue that is key to tracing the origins of Europe's now 700-million-strong population. The archaeological evidence shows that farming was introduced into Greece and southeast Europe from the Near East more than 8000 years ago, then spread west and north to the Atlantic Ocean. But did the farmers themselves move across Europe, in a migration of people and their genes? Or was the chief movement one of culture, as Paleolithic hunter-gatherers—whose ancestors arrived on the continent as long as 40,000 years ago—adopted farming?

Many studies over the past 2 decades have sought to test these hypotheses, often focusing on the DNA of modern Europeans in an attempt to trace their heritage. But the data have been conflicting. Now, a paper on page 1016 of this issue offers the first direct look at the DNA of early farmers themselves, including a sample from the Halberstadt skeleton. Anthropologist Joachim Burger and graduate student Wolfgang Haak of Johannes Gutenberg University in Mainz, Germany, and their colleagues found that many LBK farmers carry a mitochondrial DNA (mtDNA) type rarely found today, implying that they left little genetic legacy in living Europeans. The new data clash with some earlier studies, including Y chromosome analyses of living Europeans, which suggest that early farmers with roots in the Near East made a deep imprint on the European genome.

Because the Y chromosome is inherited through the male line, and mtDNA is passed down through women, some researchers now think that different genetic destinies of men and women could reconcile the data—and perhaps even the European origins debate. "A simple explanation for the difference is that indigenous hunter-gatherer females intermarried with [early] farmers," says Alexander Bentley, an anthropologist at the University of Durham, U.K.

The model of farming spread by migration, called demic diffusion, was formally proposed in 1984 by archaeologist Albert Ammerman and geneticist Luigi Luca Cavalli-Sforza. It postulates that large numbers of colonizing farmers spread across Europe, mating with some of the hunter-gatherers already there and displacing the rest through rapid local population growth. These growing populations then provided colonizers for still more movements west and north. Many early and some recent studies have supported the idea. For example, a widely cited 2002 paper by geneticist Lounès Chikhi of Paul Sabatier University in Toulouse, France, tracked Y chromosome variation in living Europeans and concluded that indigenous hunter-gatherers contributed less than 50% of the genes of modern Europeans; most genes, Chikhi concluded in the *Proceedings of the National Academy of Sciences*, came from the colonizing farmers.

But other researchers argued that there was little evidence that early farmers had undergone the kind of explosive population growth required by demic diffusion. Archaeologist Marek Zvelebil of the University of Sheffield, U.K., proposed an alternative model in which some colonization took place in certain areas—perhaps including the LBK region in central Europe—but that farming then spread mostly via local adoption rather than further movements of the original colonizers. A number of recent genetic studies have supported this model. For example, one study concluded that less than 25% of the mtDNA gene pool of modern Europeans could be traced to incoming early farmers (*Science*, 10 November 2000, p. 1080).

To try to get around this stalemate, Burger and Haak's team zeroed in on the mtDNA of ancient Europeans. The team tried to extract mtDNA from 57 individuals buried at 16 early farming sites, most from the LBK culture, and dated between 7000 and 7500 years ago. They succeeded with 24 of the skeletons. Moreover, the team found that six of the 24 skeletons had a mtDNA variant, called haplotype N1a, that is now very rare worldwide. Thus an apparently widespread mtDNA variant in early European farmers has left almost no trace on living

CREDIT: LANDESAMT FÜR ARCHÄOLOGIE SAC ISEL-WANIA, T. IAL./SAALE, GERMANY

Europeans, a finding the authors interpret as support for the cultural diffusion model.

Indeed, proponents of cultural diffusion hail the results. "It really does seem that [early farmers] must have left far fewer descendants than one might expect, given the apparent archaeological impact of the LBK at the time," says Martin Richards of the University of Leeds, U.K. Agrees Zvelebil: "This is a very important step forward, … It bypasses all the problems of extrapolation from modern DNA." But he cautions that to completely prove its case, the team should extract ancient DNA from early farmers in the Near East to see if they also have high frequencies of the N1a haplotype, as well as from hunter-gatherer skeletons in the LBK region to see if they have low frequencies.

Those who favor demic diffusion aren't yet convinced, however. "The authors are rather impatient in drawing conclusions," says Cavalli-Sforza, who thinks the results can't be properly interpreted without knowing the farmers' Y chromosome sequences too. Chikhi adds that the authors have not entirely ruled out the possibility that today's low N1a frequencies are due to chance loss of the variant. And ancient DNA pioneer Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, warns that ancient DNA studies of modern humans are notoriously unreliable because of the problem of contamination with living people's DNA. "In our experience, results from the majority of ancient human samples are irreproducible," he says.

All this leaves researchers trying to sort out the conflicting data. Most studies of mtDNA have supported the cultural diffusion hypothesis, whereas the Y chromosome data seem to favor a movement of people themselves. The idea that colonizing farmers married local hunter-gatherer women might resolve the conflict between the mtDNA and Y chromosome data and also explain the team's results, argues Bentley. "Intermarriage of farmers with indigenous women would reduce N1a in subsequent generations," he points out. Cavalli-Sforza agrees that intermarriage is a possibility, noting that men may have mated with more than one woman and that the typical LBK longhouse often had three or four hearths. "Polygynic families are a very reasonable explanation" for this architectural arrangement, he says.

For Zvelebil, the contradictory results probably indicate that neither large-scale migrations nor cultural diffusion can explain everything that happened in Europe during the adoption of agriculture. Rather, he suggests, the contribution of each of these processes probably varied from region to region: "Our prehistory was far more complicated and fascinating than either of these models allow for."

–MICHAEL BALTER

Drug Research

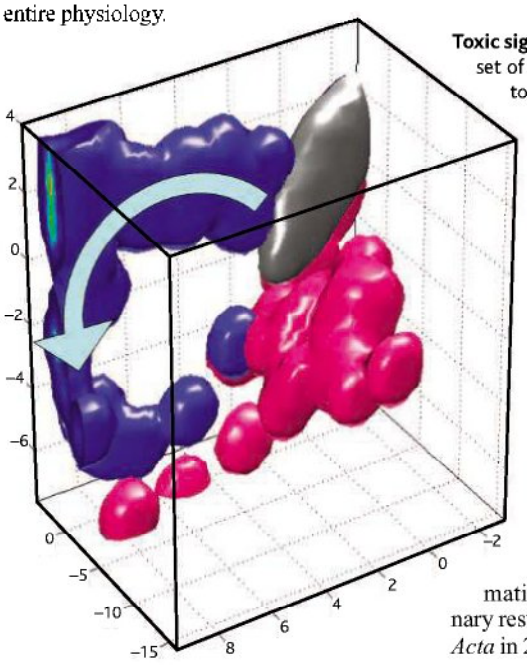
Trying to Catch Troublemakers With a Metabolic Profile

Drug discovery and toxicity research are just two areas that could benefit enormously from the use of new "metabonomic" techniques

Only one in 10 potential drug compounds ever makes it to market; the others are rejected as either too risky or ineffective. Companies have dreamed of making screening processes more efficient—and now researchers may have a way to do it. They're developing a technique based on "metabonomics," using metabolic profiles to identify toxicities rapidly and analyze the likely effects of unknown compounds. The strategy got a boost last month when several companies that had previously backed researchers at Imperial College London—including Bristol-Myers Squibb (BMS) and Pfizer—signed up to extend the work.

Metabonomics—the study of metabolic changes in urine, serum, or tissue after an organism has been exposed to a drug or other stressor—is decades old in concept. But measurement tools have become more sophisticated, making it possible to analyze data from multiple, small samples and make associations at high speed.

"It is a very powerful technology," says Bruce Carr, director of pharmaceutical candidate optimization at BMS, who has been collaborating with Imperial College researchers. Although studies suggest that companies already catch 90% of adverse effects before a drug application is submitted to the U.S. Food and Drug Administration (FDA), he believes metabolic profiling might help detect them earlier because it gives a snapshot of an organism's entire physiology.



CREDIT: ADAPTED FROM FIGURE COURTESY OF TIM EBBELS/IMPERIAL COLLEGE

Toxic signature. The CLOUDS program creates a set of references based on animals' responses to liver-toxic (blue) and kidney-toxic (red) compounds over time.

The teamwork began 5 years ago when six pharmaceutical companies including BMS and researchers at Imperial College formed the Consortium for Metabonomic Toxicology (COMET). Their goal was to develop a database of known toxins and their metabolic signatures from animal tests, to which experimental drugs with unknown toxicity could be compared. Rats were dosed at a separate facility with more than 100 toxic compounds, one per animal. The Imperial College researchers scanned urine and serum samples through nuclear magnetic resonance (NMR) spectroscopy and other technology to generate metabolic profiles of the animals.

The COMET researchers then used a computer program they had developed to assess which organs were affected. Called Classification of Unknowns by Density Superposition (CLOUDS), the software compared the NMR data—typically a hallmark signature of peaks corresponding to unknown or known metabolites—to an existing database of profiles. Tissue samples went to histology researchers for confirmation of the NMR findings. Preliminary results, published in *Analytica Chimica Acta* in 2003, demonstrated that this method

could group samples accurately by affected organ: for example, liver toxicity with up to 77% accuracy and kidney toxicity with up to 90% accuracy.

More significant, however, was the program's ability to crunch data taken at intervals in long-term studies. Researchers analyzed urine and serum samples at various times from the moment an animal was dosed with a toxin through its recovery. The program used probability calculations to assign the effects seen in each animal to the most likely toxin class and to identify when the compound caused the toxicity.

The analysis is "much more sophisticated" than any other screening tool now available, says Jeremy Nicholson, head of biological chemistry and COMET project director at Imperial College, because it can identify more simply biochemical changes that may cause pathology. (Nicholson has helped launch a spinoff in London to commercialize similar technology applied to medical diagnostics, called Metabometrix.)

Existing toxicology research methods can only examine toxic effects on one tissue type at a time. A gene-expression study, for example, yields data from a single time point in a single tissue. Moreover, changes in gene expression may not mean a net biological change. The body's homeostatic mechanisms may compensate by degrading or modifying gene transcripts. By contrast, "urine and plasma give the metabolic interaction of all tissues," Nicholson adds.

"Metabonomics has a big role to play in toxicology research," says Ian Blair, a professor of chemistry at the University of Pennsylvania. "Once you have a signature of toxicological response, you could use that as an assay for many things."

After the consortium published the initial results, each member developed its own database and technology in-house. Companies have been mum on details but have confirmed that they are building larger databases against which they can compare new compounds. Several companies also employ metabonomics to screen animals prior to an experiment to ensure that they are normal. Researchers say the technology could also be applied to clinical trials to correlate drug response to individual metabolism.

Drug companies aren't the only ones interested in metabolic profiling. In 2003, the U.S. National Institutes of Health awarded $35 million to a consortium of 18 institutions to identify, characterize, and quantify human cellular lipid metabolites. And recently Nicholson formed a coalition of scientists to establish standards for the field.

COMET's success has prompted several companies to join COMET II at Imperial College, says Nicholson. He's heading the project, which is scheduled to launch this month. The goal this time, however, is to create a "multiomics" platform that combines data from many sources, including gene and protein arrays, to reveal biochemical mechanisms.

That will be no easy task. Spectral data from a single urine sample contain thousands of peaks, the majority of which are unidentified metabolites. But the analytical tools to assign identities to the peaks are already emerging, says Nicholson. For example, his colleagues at Imperial College have a paper in press at *Analytical Chemistry* describing software that can combine data from NMR spectroscopy and mass spectrometry—similar yet complementary metabonomic techniques. And Nicholson says his colleagues have already developed a prototype system that integrates data from gene, protein, and metabolic profiles.

But whether the technology will actually help make drugs safer remains to be seen, says David Jacobson-Kram, head of pharmacology and toxicology at FDA's Office of New Drugs: "Technology can help to some extent, but perhaps our expectations are unrealistic." One potential application touted by the technology's supporters is to metabolically profile drug side effects. "Is there a metabolic profile characteristic of suicidal ideation?"—a side effect of several antidepressants—Jacobson-Kram asks. "That's a stretch."

Nevertheless, the field is young, Blair says, and the number of papers it is producing these days suggests it has begun a growth spurt.

—GUNJAN SINHA

Gunjan Sinha is a writer in Berlin, Germany.

Paleontology

Tyrannosaurus rex Gets Sensitive

Its supersized smell organs have been scaled back a bit, but new studies show that the tyrant lizard's sensory apparatus was indeed fit for a king

MESA, ARIZONA—With its powerful jaws and serrated teeth, *Tyrannosaurus rex* had fearsome tools for catching and eating prey. The lumbering carnivore also had some top-of-the-line sensory equipment, paleontologists reported here last month at the 65th annual meeting of the Society of Vertebrate Paleontology (SVP).

The new insights come from studies of bony clues to the brain, ears, and eyes of *T. rex*. They suggest that the "tyrant lizard king" had an acute sense of smell—although perhaps not as acute as some recent studies had suggested—a knack for listening as well as keeping its eyes fixed on prey, and depth perception to rival modern birds of prey. To most paleontologists, it all adds up to a talented predator. "The more we look at *T. rex*, the more sophisticated it is," says Philip Currie of the University of Alberta in Edmonton, Canada.

You can't hide. *Tyrannosaurus rex*'s keen senses of smell, hearing, and vision helped it take down prey or snap up carrion.

CREDIT: JIM ZUCKERMAN/CORBIS

T. rex was first unveiled and named 100 years ago by the legendary paleontologist Henry Fairfield Osborn of the American Museum of Natural History in New York City. Ever since, *T. rex* has been famous for its staggering dimensions—ranging up to 12 meters and perhaps as much as 7 tons—and its highly modified skeleton. Several of those distinctive features, such as the shrimpy arms and the bone-crushing teeth, led some researchers to propose that *T. rex* was prima-

rily a scavenger. That case was bolstered in 1999, when computed tomography (CT) scans revealed that the *T. rex* named Sue had enormous olfactory bulbs (*Science*, 9 June 2000, p. 1728)—a specialization that would presumably have helped it catch the scent of a dead dinosaur in the distance.

To some paleontologists, the gargantuan olfactory bulbs were difficult to swallow. One group of researchers—François Therrien of the Royal Tyrrell Museum of Palaeontology in Drumheller, Canada, and Farheen Ali and David Weishampel of the Johns Hopkins University School of Medicine in Baltimore, Maryland—decided to see what the olfactory bulbs in *Tyrannosaurus*'s closest living relatives, birds and crocodiles, could reveal about their long-gone cousin.

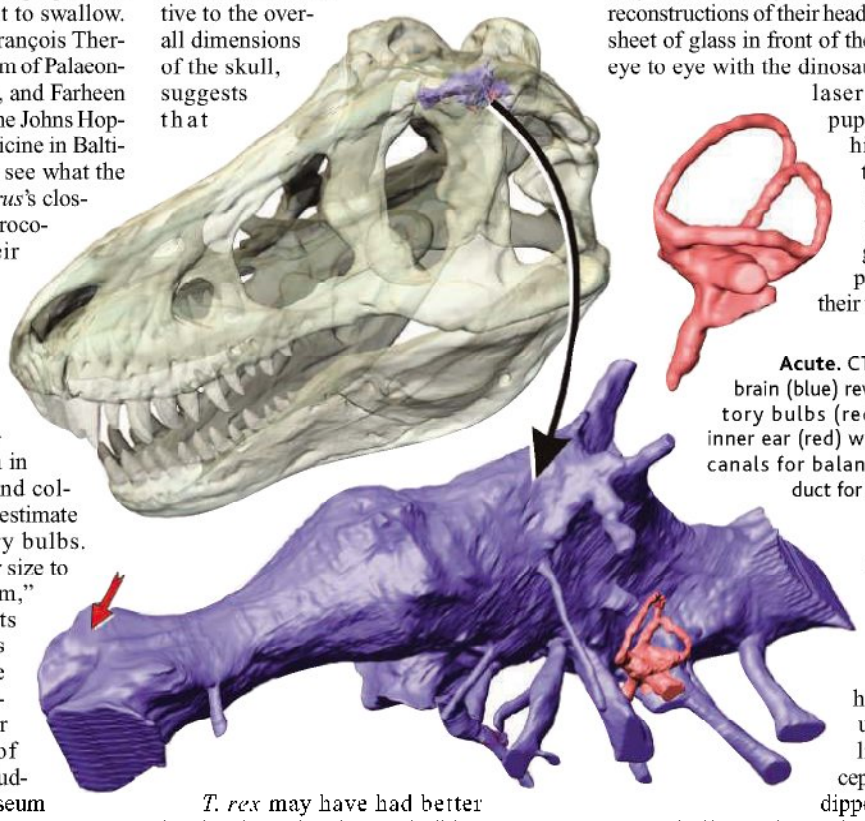
In both groups, the olfactory bulbs rest against a trough on the upper part of the braincase and are bounded toward the front of the head by a septum. By locating bony traces of this septum in *T. rex* braincases, Therrien and colleagues could more accurately estimate the position of the olfactory bulbs. "This would have limited their size to no more than that of a plum," Therrien says. Paleontologists had thought the olfactory lobes extended further forward inside the head. "I think they're probably right," says Christopher Brochu of the University of Iowa in Iowa City, who had studied Sue while at the Field Museum in Chicago.

Still, *T. rex* did very well by its nose. The relative size of the bulbs—their width compared with the width of the cerebral hemispheres—was the highest of seven dinosaurs examined, including other tyrannosaurids and smaller, more birdlike dinosaurs.

Therrien speculates that the acute sense of smell could have been used to track prey, locate putrefying carcasses, or help males patrol territory for rivals. Greg Erickson of Florida State University in Tallahassee cautions that it's not straightforward to link organ size to sensory acuity, as sense of smell is also determined by features such as the density of neurons. "We need more [comparative] studies … so we can make sense of this," he says.

In the meantime, another study described at the meeting matched Therrien's results on the size of the olfactory bulbs. Lawrence Witmer and Ryan Ridgely of Ohio University College of Osteopathic Medicine in Athens put several *T. rex* skulls into a CT scanner. By examining features preserved in the skull, including fossilized evidence of nasal tissue in front of the olfactory bulbs, Witmer found that the bulbs were roughly walnut-sized.

Witmer's study extracted clues to other sensory abilities. For example, he resolved the bones that surrounded the so-called cochlear duct of the inner ear, which helps turn sounds into nerve signals. The length of these bones, relative to the overall dimensions of the skull, suggests that *T. rex* may have had better hearing than other theropods did.



Acute. CT scans of *T. rex*'s brain (blue) reveal sizable olfactory bulbs (red arrow) and an inner ear (red) with long, delicate canals for balance and cochlear duct for hearing.

The inner ear can also reveal aspects of an extinct animal's posture and sense of balance (*Science*, 31 October 2003, p. 770). Thanks to the CT scans, Witmer could resolve the bony labyrinth of the inner ear with its trio of semicircular canals, oriented at right angles to one another. These once contained fine hairs that sensed the motion of fluid, helping the brain know how the body was oriented and which way it was moving. In modern creatures, the larger the loop of the semicircular canals, relative to head size, the more agile they tend to be.

T. rex turned out to have surprisingly long canals. "You might not expect a large animal to have quick movements," Witmer says. He suspects that the primary purpose of the canals was not gymnastics but helping *T. rex* keep its head and eyes fixed on prey. That's not all the canals reveal. In modern animals, the orientation of the lateral canal relative to the skull correlates with how they tend to hold their heads while alert. *T. rex* apparently kept its head dipped down about 5° to 10°. For tall animals with long snouts, such as *T. rex*, tilting the head downward can help them better see what's directly ahead.

Kent Stevens of the University of Oregon, Eugene, has come to a similar conclusion about *T. rex*'s vision, which again places it at the top of its class. He reconstructed the visual abilities of *T. rex* and six other predatory dinosaurs by working with sculpted reconstructions of their heads. After placing a sheet of glass in front of the busts, he stood eye to eye with the dinosaurs and shined a laser at each fake pupil. This allowed him to map onto the glass the entire area from which the laser glinted off the pupils, tracing their visual field.

T. rex, with its forward-facing and widely separated eye sockets, turned out to have great binocular vision and, likely, depth perception. When *T. rex* dipped its head about 10°—similar to the angle of the alert posture that Witmer estimated—it would have maximized the width of its binocular field of view at 55°, as good as that of hawks, Stevens says. That's not quite as good as those of the highly birdlike dinosaurs, such as *Troodon*, but it exceeds that of other adult tyrannosaurids. The research, which Stevens presented at an SVP meeting several years ago, is in press at the *Journal of Vertebrate Paleontology*.

To Stevens, the degree of depth perception, hearing, and sense of smell point in one direction: a top predator. In contrast, Jack Horner of the Museum of the Rockies in Bozeman, Montana, is sticking with his idea of where *T. rex* got its meals. "I think this olfactory business is very supportive of the *T. rex*–as-scavenger hypothesis." Others say it's more likely that *T. rex* wasn't a picky eater. "If it can smell a carcass a mile away, it can also smell a herd of hadrosaurs from a mile away," says James Hopson of the University of Chicago in Illinois. "I don't think it would have preferred one over the other."

–ERIK STOKSTAD

RANDOM SAMPLES

Edited by Constance Holden

The State of Africa's Lakes

The U.N. Environment Programme has assembled a dismaying picture of the degradation of Africa's 677 lakes. Last week, it introduced a new Atlas of African Lakes at the World Lake Conference in Nairobi, Kenya. Above, satellite images show Lake Songor Lagoon in Ghana, which has lost volume and biodiversity between 1990 (left) and 2000 (right) due in part to salt mining.

Hellenistic Engineering

Last month in Athens, scientists unveiled a working model of a mysterious instrument discovered a century ago in the ruins of a 2000-year-old Greek shipwreck.

Found as a crusted bronze mass in the cargo of a ship that sank off the island of Antikythera, the instrument, dubbed the "Antikythera Mechanism," was a jumble of gears and dials encased in a wooden box.

Yale University science historian Derek de Solla Price puzzled for many years over the instrument. After x-raying it, he concluded in 1974 that it was designed to compute solar and lunar cycles. He described some 30 bronze gears that required a differential turntable to coordinate them—which would have been a revolutionary technology for the time.

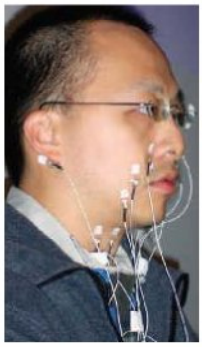
In 1989, engineer Michael Wright, now at Imperial College London, and Sydney University computer scientist Allan Bromley applied more advanced imaging technology to determine the level of each wheel and gear within the mass. They showed that Price's inclusion of a differential gear was incorrect. Bromley's death interrupted the work, but in 2002, Wright started again on a reconstruction. His complete working model, unveiled at the Second Conference on Ancient Greek Technology in Athens, demonstrates that the mechanism included a complete planetarium, showing the orbits, or epicycles as the Greeks called them, of not only the sun and moon but also the five planets known to the Greeks: Venus, Mars, Jupiter, Saturn, and Mercury. The instrument shows that intricate geared mechanisms were "an accepted element of Hellenistic technology," says Wright.

Mystery planetarium reconstructed.

Latest in Translation

Grad student Stan Jou was mouthing Mandarin Chinese, but no sounds issued from his mouth. Instead, a robotic voice from a speaker spoke for him, using inputs from electrodes glued to his cheeks and throat. The words, in English or Spanish, were part of a press conference last week at which computer scientist Alex Waibel of Carnegie Mellon University in Pittsburgh, Pennsylvania, and others showed off their latest toys for speech recognition and translation.

The electrodes on Jou's face picked up movements of his face and throat muscles. Software turned them into words, which were then translated. So far, the system can only recognize about 15 phrases. But Waibel predicts that someday people will be able to have face-to-face conversations in alien tongues without the sounds of their original words getting in the way.



The researchers are also developing goggles displaying simultaneous translations of a talk. And they've built directed speakers that can pinpoint a person in a crowd and deliver a translation as if it were being whispered in the ear. Waibel's software for translating spoken language is some of the best in the world, says Satoshi Nakamura of the Advanced Telecommunications Research Institute in Japan, but he doubts such a program will make it to the marketplace in this decade.

Some of this technology could require more-or-less permanent attachments to the listener. But, says Waibel, "I think someday people will accept having a few electrodes implanted in their cheek."

CREDITS (TOP TO BOTTOM): COURTESY OF NASA; COURTESY OF NASA; ALEX WAIBEL; MICHAEL WRIGHT

Who's No. 1?

Britain's Royal Society launched two polls this week—an online one for the public and one for scientists—on whether Einstein or Newton is "the greatest scientific heavyweight of all time." Results will be announced at an "Einstein vs. Newton debate" in London on 23 November.

According to Royal Society vice president Martin Taylor, the society is hoping the contest will inspire British students, whose interest in physics has "reached a historical low." Vote at www.royalsoc.ac.uk.

Edited by Yudhijit Bhattacharjee

ON CAMPUS

Team effort. Virginia Tech materials scientist Brian Love and his students are helping the college's star running back stay in the limelight.

On 7 October, Cedric Humes, the starting tailback on the Blacksburg university's undefeated and number-3-ranked football team, broke his right forearm in a game against Marshall University. The team's athletic trainer, Mike Goforth, called Love (inset) to see if he could devise a cast better than the padded fiberglass model that would have left Humes unable to feel the football in his arms and increased his likelihood of fumbling. Love challenged his students to come up with an alternative. Using moldable carbon fiber plastics often used for pelvic fractures, Humes's doctors fashioned a brace that covered just the outside of the athlete's forearm.

Three weeks after the injury, Humes was back on the field, carrying the ball 13 times without a fumble to help the Hokies to a 30–10 victory over Boston College. The splint is so strong, "I think you could drive a car over it," Goforth says. Humes plans to thank Love and his students with a signed game ball.

A LIFE IN SCIENCE

Total immersion. Nanotech pioneer Richard Smalley, who died 28 October, did not view any task as beneath him, says chemist Jim Heath of the California Institute of Technology in Pasadena. "Even when he was famous, he would sweep the floors if he thought it would help to get the science done. Once, somebody dropped a screwdriver into a huge vacuum chamber—the same one that was used for the discovery of C_{60}. The screwdriver handle dissolved in the oil [at the bottom of the chamber], so the chamber had to be cleaned out. [It] was about 10 feet [3 m] high and could only be accessed through a hole in the top. Rick and I had to strip down to our underwear and take turns holding each other by the ankles and lowering the other into the chamber to clean it out."

MISCONDUCT

Pressure to publish. A former postdoc who falsified images in a paper has been banned from receiving U.S. research funding for 3 years. The Department of Health and Human Services' Office of Research Integrity in September found Xiaowu Li guilty of scientific misconduct for passing off images of mouse melanoma cells as human pancreatic cancer cells in a paper published online March 2004 in *Carcinogenesis*.

Li was working under cancer researcher Daniel Ramos at the University of California, San Francisco. Ramos says he was unaware of the publication, which Li wrote with a group of researchers in China, and was initially upset that he hadn't been asked to be a co-author. But once he recognized the false images, which were taken from his own lab, he contacted university officials. By then, Li had left the university to work at China's Southwestern Hospital in Chongqing, where some of his co-authors are based.

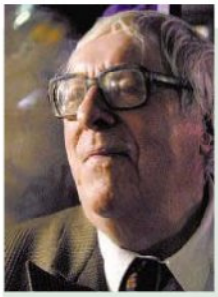
Ramos says the results of other experiments he performed with Li appear to be valid. He says Li told him during the investigation that the pressure to compile an impressive research record drove him to commit misconduct. (*Science* was unable to contact Li.) "It kills me," Ramos says. "He was good—he didn't need to do something like this."

NONPROFIT WORLD

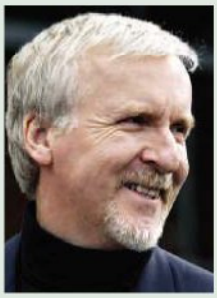
Fueling science. John Browne, an oil magnate with an interest in research, will be the next president of the British Association for the Advancement of Science.

Now group chief executive of British Petroleum, Browne oversaw the merger of BP and Amoco in 1998 and has drawn attention to climate-change risks. Last year, he wrote that "global warming is real and ... we should start taking the small steps to reduce carbon dioxide emissions today." (BP says it cut greenhouse gas emissions by 10% between 1998 and 2001.) Browne, 57, who has an undergraduate degree in physics and a master's degree in business, will take the helm of the 174-year-old association next September, succeeding Frances Cairncross.

AWARDS



Inspiring tales. In the quest to explore Earth and other planets, firing imaginations may be as important as firing rockets. That's why the Planetary Society is honoring two nonscientists at its 25th anniversary celebration this week: writer Ray Bradbury, who has transported readers to the planets in *The Martian Chronicles* and other works, and filmmaker James Cameron, who directed the blockbuster *Titanic* and has taken viewers for otherworldly tours of the ocean floors in his documentaries *Ghosts of the Abyss* and *Aliens of the Deep*.



The 85-year-old Bradbury will receive the Thomas O. Paine Memorial Award for the Advancement of Human Exploration of Mars. Previous winners include members of the Mars Pathfinder and Mars Global Surveyor missions. "There was a lot of intelligent imagination in what he wrote," says Wesley Huntress, president of the society and director of the Geophysical Laboratory of the Carnegie Institution of Washington, D.C., who credits Bradbury's writings for inspiring him to become a space scientist. Cameron, 51, will receive the society's inaugural Cosmos Award for Outstanding Public Presentation of Science.

Got any tips for this page?
E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): DAVID KNACHEL/VIRGINIA TECH; INSET: KAREN GILBERT/VIRGINIA TECH; AP/STF; A. VO PALTZER; R. CUT-2S/LLC; CELANO

subject to significant bias. Authors who identify appropriate reviewers are more likely to be well established in their fields than authors who do not, reflecting not only on the quality of their research but also on their ability to gauge whether a particular manuscript is of sufficient standard for a given journal. Furthermore, it is unsurprising that authors who exclude specific reviewers are more likely to be successful, particularly if the reason for exclusion is that the potential reviewer is a perceived competitor, suggesting that the manuscript in question describes relevant and highly publishable research. I have no doubt that personal prejudice has occasionally superseded scientific judgment in the peer-review process. However, like David Nordstrom, I prefer to believe that quality of research is the prevailing factor in the vast majority of cases. As for my own rejected manuscripts? In the words of Franklin P. Jones: "Honest criticism is hard to take, particularly from a relative, a friend, an acquaintance, or a stranger."

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IN HIS ARTICLE "SUGGESTING OR EXCLUDING reviewers can help get your paper published" (News of the Week, 23 Sept., p. 1974), D. Grimm examines a potential problem with peer review in science. When authors submit papers and recommend that particular scientists either referee or be excluded from the pool of referees for their paper, their paper is (i) more likely to be accepted and (ii) less likely to be rejected.

Nevertheless, there may be a bias in the sample of papers with suggestions of referees. Such authors may generally be better researchers, suggesting that their papers both get published and cited at a higher rate than other scientists. This hypothesis could easily be tested by comparing the rates of citations to papers that were refereed by scientists suggested by the authors with the rates of citations to papers that were refereed by scientists chosen by the editor and editorial board alone. If the first set of papers end up being cited more frequently than the second, then the first set are probably more important and thus deserve to be accepted for publication at a higher rate. If so, the peer-review process is working as it should. But, if we find that the latter set of papers are cited more frequently than the former, then the practice of allowing authors to recommend referees should be discontinued.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Correcting Temperature Data Sets

WE AGREE WITH C. A. MEARS AND F. J. WENTZ ("The effect of diurnal correction on satellite-derived lower tropospheric temperature," Reports, 2 Sept., p. 1548; published online 11 Aug.) that our University of Alabama in Huntsville (UAH) method of calculating a diurnal correction to our lower tropospheric (LT) temperature data (v5.1) introduced a spurious component. We are grateful that they spotted the error and have made the necessary adjustments. The new UAH LT trend (v5.2, December 1978 to July 2005) is 0.123 K/decade, or 0.035 K/decade warmer than v5.1. This adjustment is within our previously published error margin of ±0.05 K/decade (*1*).

We agree with S. C. Sherwood *et al.* ("Radiosonde daytime biases and late–20th century warming," Reports, 2 Sept., p. 1556; published online 11 Aug.) that there are significant, progressively colder biases in stratospheric radiosonde data, as we and others have noted (*1*, *2*). We further agree that many daytime radiosondes are plagued by spurious cooling in the troposphere as well (*3*). However, there are also instances in which spurious warming occurs in both day and night soundings. Such a circumstance is not properly accommodated by the day-minus-night (DMN) procedure, a possibility mentioned by Sherwood *et al.* but not specifically addressed. For example, when the Australian/New Zealand network, prominent in the Southern Hemisphere in Sherwood *et al.*'s Report, switched instrumentation from Mark III to Vaisala RS-80, both day and night warmed approximately 0.4 K [(*3*), updated], with tropospheric night readings warming more than day readings. On the basis of this relative difference, the DMN method assumes that a correction for spurious cooling should be applied, when in fact the real error is large and of the opposite sign.

DMN values are useful indicators for pointing out radiosonde changes, but they are often not useful in assessing magnitudes and in this case overestimate the trend. Further, the DMN-adjusted tropospheric trend for 1958–97 of 0.253 K/decade for the 75% of the globe south of 30°N is more than 2.5 times that of the surface (0.092 K/decade) and thus very likely to be spuriously warm. [Note that B. D. Santer *et al.* ("Amplification of surface temperature trends and variability in the tropical atmosphere," Reports, 2 Sept., p. 1551; published online 11 Aug.) indicate a ratio less than 1.4.] Direct, site-by-site comparisons between radiosondes and UAH LT data at 26 U.S.-controlled stations (nighttime only) from tropics to polar latitudes yield a difference in trends of less than 0.03 K/decade, showing consistency with the more modest UAH LT trends (*1*) [(*3*), updated through 2004].

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Response

ONCE WE REALIZED THAT THE DIURNAL CORrection being used by Christy and Spencer for the lower troposphere had the opposite sign from their correction for the middle troposphere sign, we knew that something was amiss. Clearly, the lower troposphere does not warm at night and cool in the middle of the day. We question why Christy and Spencer adopted an obviously wrong diurnal correction in the first place. They first implemented it in 1998 in response to Wentz and Schabel (*1*), which found a previous error in their methodology: neglecting the effects of orbit decay.

CARL A. MEARS AND FRANK J. WENTZ
Remote Sensing Systems, 438 First Street, Suite 200, Santa Rosa, CA 94501, USA.

Reference
1. F. J. Wentz, M. Schabel, *Nature* **394**, 661 (1998).

Response

WE ARE HAPPY THAT CHRISTY AND SPENCER do not dispute our main conclusion, that changes in systematic error in the radiosonde record are comparable to expected trends. However, their characterization of our work as a "DMN method" downplays the key fact that we have identified and quantified a source of error. One need not assume that nighttime readings are absolutely correct to recognize that, because they do not contain this error, they are less likely to differ from the truth than are daytime data. Any remaining errors affecting both times of day are much more difficult to quantify, and estimates of their magnitude will be sensitive to the assumptions and proce-

dures followed. We welcome Christy and Spencer's efforts to estimate these additional errors and look forward to seeing details published as to how they arrived at the numbers quoted and what they found at other stations.

There is growing evidence, however, that the net result of all adjustments will indeed lead to increased warming, contrary to their assertion. First, all published homogenization efforts have led to increased warming (*1*–*3*); although weaker than what we found, this is probably because previous efforts went only after the "biggest fish" and/or suffered from other difficulties. Second, a new and independent study (*4*) strongly suggests that the spurious cooling trends in the stratosphere extend into the troposphere, in accord with our findings and as suggested previously (*1*). The implication by Christy and Spencer that spurious warmings (which have been documented in the other studies as well) somehow compensate for daytime heating effects in the troposphere, but not in the stratosphere, will require clear support from the data and careful scrutiny of methods. The agreement noted by Christy and Spencer at U.S. stations is encouraging but does not guarantee agreement in the Tropics [and mustn't this previously reported agreement have been affected by the recent revision to their method (*5*)?].

The trend noted by Christy and Spencer south of 30°N is a misleading statistic that mixes up two parts of the globe whose situation is very different. In the Tropics, sampling is adequate and we find a large error that brings the data closer to what is expected. South of 30°S, on the other hand, sampling is far from adequate, and radiosonde trends have always been erratic, with or without the relatively modest correction implied by our work.

Quite apart from this, it is hard to believe that Christy and Spencer would argue that a data set showing the "wrong" amount of warming must therefore be flawed. If that were a valid argument, their own satellite analysis would have been discarded years ago.

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Causation, Vioxx, and Legal Issues

IN THE ARTICLE ON THE FIRST VIOXX TRIAL, "Vioxx verdict: too little or too much science?" (News Focus, 2 Sept., p. 1481), A. Lawler writes that one commentator attributed the jury's verdict for the plaintiff to the evidence about Merck covering up the problems with its drug. This occurred in a case that most observers thought was one of the weakest ones on individual causation.

In law (as in science), causation is a matter independent of culpability. A drug may innocently cause harm, and the most heinous corporate actions may, through serendipity, not result in harm. Yet the Vioxx verdict appears to be a reprise of what occurred with the drug Bendectin and silicon gel breast implants, in which juries relied on evidence of corporate wrongdoing to reach verdicts that the evidence of causation would not justify (*1*, *2*).

Remarkably, the success of plaintiffs with juries continued in the Bendectin litigation even after the science tending to exonerate the drug became more robust (*3*).

For the most part, courts corrected those errors in Bendectin (which spawned the famous *Daubert* decision, requiring federal judges to more aggressively screen expert testimony) and in breast-implant litigation. Merck may not benefit from the same judicial intervention. There is, after all, pretty good evidence that Vioxx has caused a substantial number of heart attacks, and those plaintiffs are queuing up for their turn. The first case appears to have ridden on their anticipated coattails.

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Illusory Statistics

THE REPORT BY S. NEE *ET AL.* "THE ILLUSION OF invariant quantities in life histories" (19 Aug., p. 1236) demonstrates that empirical support for the presence of invariant ratios in life-history traits is based on spurious correlations. Unfortunately, their example is just one of many: Spurious correlations have been repeatedly raised as statistical proofs for concepts as varied as the energetic costs of reproduction (*1*), rates of morphological evolution (*2*), and estimates of forest biomass (*3*).

The repeated and blind application of discredited statistics is a stumbling block to

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the advancement of science. The mere possibility that a statistical artefact could form the empirical base from which a field of evolutionary biology has grown is a sign that something is wrong. It is symptomatic of a larger issue in the biological sciences: To be a good biologist, you must also be a competent statistician, but many are not. To quote one viewpoint recently expressed, "If you can't understand enough statistics to interpret the data from your own experiments, then you probably don't deserve a Ph.D. in ecology" [(*4*), p. 49].

Spurious correlations in biological data are a commonly described phenomenon—Pearson first proved their existence to evolutionary biologists more than a century ago (*5*). One hundred and eight years on, Nee *et al*. have shown that this simple statistical message is finally sinking in.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "A glass ceiling for Asian scientists?" by J. Mervis (28 Oct., p. 606). The article incorrectly implied that an invitation to Liqun Luo to join the program committee of the Society for Neuroscience came in response to a letter questioning the society's commitment to providing opportunities for Asian-American scientists. The appointment occurred before the letter was submitted, as part of the society's normal process of replacing committee members. In addition, the article misspelled the first name of Irwin Levitan, who chairs the society's committee on committees.

AAAS News and Notes: "2006 Annual Meeting: Grand Challenges, Great Opportunities" (28 Oct., p. 635). Two lines were missing from the the last paragraph in column 1 on page 635. The missing text is "Altogether, there will be more than 200 symposia, lectures, seminars, and other sessions. For more about the program and registration, see www.aaasmeeting.org." The text is correct in the online version.

News of the Week: "Six women among 13 NIH 'Pioneers' " (30 Sept., p. 2149). The first name of Pehr Harbury, chosen for the 2005 Director's Pioneer Award by the National Institutes of Health, was misspelled in the picture caption that accompanied the story.

Policy Forum: "Pathogen surveillance in animals" by T. Kuiken *et al.* (9 Sept., p. 1680). In reference (*16*), part E of the figure was incorrectly attributed to the Australian Broadcasting Corporation; the photograph is from Reuters.

PUBLIC HEALTH

A Vaccine Disaster and Its Fateful Shadow

Olen Kew

Five decades ago in Ann Arbor, Michigan, Thomas Francis made a momentous announcement: the polio vaccine developed by Jonas Salk and his team worked. The news was hailed as one of the greatest triumphs of science, medicine, and public health. Development of a safe and effective polio vaccine, through the leadership of the National Foundation for Infantile Paralysis and its March of Dimes campaigns, reaffirmed the spirit of volunteerism in the United States and restored public confidence in vaccines following two decades of disaster. Church bells rang throughout the land in celebration, and Jonas Salk enjoyed celebrity unprecedented for a medical scientist. As Paul Offit vividly describes in *The Cutter Incident: How America's First Polio Vaccine Led to the Growing Vaccine Crisis*, the announcement came at a time of devastating polio epidemics that paralyzed tens of thousands of children each year, a time when Americans' fear of polio was surpassed only by their fear of nuclear war. The new vaccine was promptly licensed, and communities were mobilized to deliver millions of doses to children throughout the country.

The Cutter Incident
How America's First Polio Vaccine Led to the Growing Vaccine Crisis
by Paul A. Offit

Yale University Press, New Haven, CT, 2005. 250 pp. $27.50. ISBN 0-300-10864-8.

Within three weeks, triumph turned to tragedy as reports streamed in of polio cases among recently immunized infants and children, principally from the western states. The clinical and epidemiologic findings clearly implicated the polio vaccine and narrowed the risk to specific lots produced by Cutter Laboratories of Berkeley, California, one of the five American producers of the vaccine. In his gripping narrative, Offit (an immunologist and pediatrician at the Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine) recounts the terrible dilemma faced by public health officials as they urgently sought a way to prevent further cases while not undermining public confidence in the polio vaccine just as the peak transmission season for circulating polioviruses was beginning. Because the regulations governing vaccine production were at the time quite limited, the officials had essentially no knowledge of the problems that Cutter and other manufacturers had encountered in producing polio vaccine lots free of infectious virus. Crucial decisions were made on the basis of very limited information. A consequential backdrop to these events was widespread skepticism about Salk's polio vaccine among leaders in the scientific community—skepticism fueled by a mixture of intense personal rivalry and the view that the attenuated polio vaccine then under development offered a more technically elegant, and potentially more broadly applicable, solution. In the wake of the Cutter tragedy, some leading scientists even asserted that Salk's theories and methods were fundamentally flawed and that production of an inactivated polio vaccine free of infectious virus was theoretically impossible.

Ready for the rollout. Drawing on stockpiles of bottles (such as these photographed in New Jersey in January 1955), the five manufacturers distributed more than 4.8 million doses of polio vaccine in the first three weeks after the April 1955 licensing of the vaccine.

Subsequent events have vindicated the Salk vaccine, because many millions of doses were produced and administered after 1955 without incident. The availability of an effective polio vaccine in 1955 saved tens of thousands of children in the United States, Canada, and Europe from lifelong paralysis and demonstrated the feasibility of widespread immunization to control polio. Immunization with the live, attenuated oral polio vaccine of Sabin, licensed in 1961, completed the task already well advanced by use of Salk's vaccine, and the last pockets of indigenous poliovirus transmission were eliminated in the United States by the 1970s. Building upon the successful elimination of polio from developed countries, the World Health Organization established the Global Polio Eradication Initiative in 1988 to fulfill the promise of a polio-free world first envisioned in 1955. In this global effort, the more easily administered Sabin vaccine has been the primary weapon against polio, but many countries, including the United States, have returned to the Salk vaccine to maintain a polio-free status.

Offit's book is a comprehensive and readily comprehensible account that seamlessly moves from historical narrative through technical exposition, mystery thriller, courtroom drama, and legal review to social commentary. In this last aspect, Offit presents his most compelling message: that the Cutter incident lies at the root of our current vaccine crisis. He recounts how thoughtful jurors, following a judge's strict instructions, reluctantly found Cutter liable for financial damages even though they believed that Cutter was not negligent in the production of polio vaccine. He then traces how the principle of liability without negligence was aggressively expanded in subsequent court decisions to liability even for the manufacture of safe products. Echoes of the Cutter decision still reverberate today in the diminishing number of vaccine manufacturers, the high prices for vaccines in the United States and other developed countries, and the insufficient current supply of influenza vaccine as we face a possible pandemic.

The Cutter Incident offers a concise and thoroughly documented account (well illustrated with rare period photos) of a medical tragedy and its continuing consequences. Offit presents a powerful case for a far more enlightened approach to the development and use of lifesaving vaccines.

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PHYSICS

A Comical Look at Real Physics

Sam Kean

James Kakalios is a physicist who knows how to shrink the separation between physics and play. While his research is directed toward understanding the properties of amorphous semiconductors, he also investigates problems that can be grasped at a glance. He has studied the "Brazil nut problem"—why the large, heavy nuts seem to defy gravity and rise to the top when you shake a can of mixed nuts. And he has piled up sand to see how steep the slopes can get before the grains start to spill down the sides. Thus, it comes as no surprise that in *The Physics of Superheroes* Kakalios offers a droll but sincere look at what Superman and Spider-Man can teach about physics. Granting the one-time "miracle exceptions" that give superheroes their powers in the first place, it turns out they can teach quite a lot.

In the introduction, Kakalios describes his motivation for writing the book. He reports overhearing a conversation between two students after a physics exam that had evidently not gone well for them. One complained to the other (in the author's cleaned-up version), "I'm going to bleeping buy low, and bleeping sell high. I don't need to know about no bleeping balls thrown off no bleeping cliffs." Kakalios notes two things we can learn from this complaint: "the secret to financial success" and "that the examples used in traditional physics classes strike many students as divorced from their everyday concerns."

Surprisingly, when Kakalios introduced superhero-related homework, his students at the University of Minnesota stopped complaining. He found that problems about Magneto and the Flash never struck them as unrealistic, and comic books proved an excellent way to teach the topic. A lifelong comic-book junkie, Kakalios developed a freshman seminar he titled "Everything I Know About Science I Learned from Reading Comic Books." *The Physics of Superheroes* builds on that popular course. (Disclosure: I studied physics at Minnesota and had a passing acquaintance with the author.)

The book follows the familiar path of introductory college physics classes: it starts with Newtonian mechanics, moves to the conservation laws of energy and thermodynamics, veers into electricity and magnetism, and ends with the modern physics of relativity and quantum mechanics. But the examples for each topic spring directly from the comics, and the book reproduces dozens of panels that depict various scenes. For instance, we spot the Man of Steel leaping over a building in a single bound, which prompts Kakalios to ask, "With what speed did Superman have to jump?" Ant-Man—a superhero who shrinks to miniscule sizes—appears in a series of chapters and raises a host of interesting questions. Would Ant-Man be able to speak or hear, given that his vocal cords and eardrums have shrunk, too? Is the scene where he punches his way out of a vacuum bag realistic, given that his tiny arms are much less powerful levers than a full-sized human's?

The Physics of Superheroes
by James Kakalios
Gotham, New York, 2005. 384 pp. $26, C$36. ISBN 1-592-40146-5.

Perilous to the tiny. In *Tales to Astonish* #48, Ant-Man is helpless in a partially filled bathtub.

Three extra sections follow the main text. The first offers a list of cases where comic books clearly got their physics wrong—for instance, according to Newtonian action and reaction, the power beams from Cyclops's glasses should snap his head violently backward, but they never do. The second presents a fairly typical paean about the joy and elegant power of science to study the world. In the third, "Ask Dr. K," Kakalios provides the final word on such questions as whether Wolverine's claws can shred Captain America's shield and who is the most physically realistic superhero (an easy one).

In addition to discussing the physics, Kakalios often digresses into the history of comic books, where he differentiates between the golden age (1940s) and the silver age (late 1950s and 1960s). He also outlines some famous comic-book debates. For instance, when the Green Goblin pushed Gwen Stacy off a bridge, what actually killed her? The fall or Spider-Man's attempt to stop his girlfriend's descent too quickly? (Kakalios blames Spider-Man.) As someone who has never read a comic book in his life, I found these asides a diverting respite from the science.

Kakalios infuses the book with humor. He has let in some real groaners, but nothing worse than can be expected from the worlds of science fiction and comic books. More troubling are his lapses into dense and unfriendly prose. Unfortunately, more than a few passages read like the following:

> In this situation a force will be applied to the charges in the moving wire that will induce them to flow. By dragging the wire through the external magnetic field, we convert the physical energy involved in moving the wire into a form of electrical energy manifested by the electrical current.

This reminded me of classical Greek texts on geometry or physics, which contain statements like, "As is the ratio of the whole to twice the whole, so is the ratio of that double to four times the whole." If you already know what the passage is talking about—$^1/_2 = {}^2/_4$—then the wording seems quite clear. But if the material is unfamiliar, the text is obscure. Similarly, those with a technical background can skim Kakalios's dense passages as a refresher, but neophytes may be left with a headache. Kakalios intended the book for general readers. He should be commended for avoiding too many equations; nonetheless, there are still a few dizzying pages.

The trouble spots, however, only occasionally cloud the author's entertaining account. Most of his explanations are lucid and smooth. In the end, Kakalios demonstrates that if one suspends belief and accepts that radioactive spiders or mutant arch-criminals exist, much of the physics in comic books is surprisingly reliable. From Newtonian mechanics to the quantum world, comic-book authors generally know what they're talking about. And with *The Physics of Superheroes* as a guide, now so will their readers.

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WMD Sensors—Search and Seizure

Don Prosnitz

On 11 June 2001, the Supreme Court held in *Kyllo* v. *United States* (*1*) that law enforcement's warrantless viewing of a private residence with advanced sense-enhancing technology, an infrared camera, was unconstitutional. The Fourth Amendment states, "[T]he right of the people to be secure in their persons, houses, papers, and effects, against unreasonable searches and seizures, shall not be violated, and no Warrants shall issue, but upon probable cause…." Three months later, terrorists attacked the World Trade Center and Pentagon. The next month, anthrax was mailed to members of Congress. The science and technical community was mobilized to design and deploy advanced sensors not in general public use capable of detecting chemical, biological, and nuclear weapons. Virtually all operational scenarios for detecting weapons of mass destruction (WMDs) preclude obtaining either prior consent for a search or a warrant.

Terrorists and WMDs will be with us for the foreseeable future. New technology to combat these threats should not be developed in a legal vacuum. Balancing security and civil liberties is a shared burden. Courts, legislatures, citizens, and the technical community must all participate.

In light of *Kyllo*, how can searching for WMDs be made compatible with the Fourth Amendment? A search meant physical trespass until 1967, when the Supreme Court held that the Constitution protects people, not places, and that the government may not violate an individual's reasonable expectation of privacy. When defining a reasonable expectation of privacy, the Court has considered the location of a search (homes are the most inviolate), activity revealed by the search [intimate details are inherently private (*2*)], if proactive actions were taken to protect privacy, the objective of the search [there is no expectation of privacy in contraband (*3*, *4*)], and technologies used to conduct the search [there may be an expectation of privacy if a sensor is not in general public use (*5*)].

Detaining or even delaying an individual by conducting a WMD search at a roadblock is a seizure. An individual or possessions may be seized without a warrant given reasonable, articulable suspicion of criminal activity, but undue delay [e.g., 90 minutes to locate a drug-sniffing dog to justify probable cause (*4*)] may lead to a later determination that the seizure was unreasonable.

To permit suspicionless, random seizures (e.g., highway checkpoints), courts have balanced (i) the gravity of public concern served by the seizure, (ii) the effectiveness of the seizure in advancing the public interest, and (iii) the severity of interference with individual liberty (*6*). The threat of WMDs may be so great that it will trump all other factors. However, at least one court held that a "yellow alert" is not enough to justify nonspecific searches (*7*). Because quantitatively measuring deterrence against terrorism is problematic, judgments about effectiveness will likely remain with government officials and will be adjudicated by the courts. Interfering with individual liberties is usually interpreted to mean the length of seizure, extent of physical intrusion, intimate details revealed, area searched, and public humiliation.

Developers of WMD detectors cannot anticipate future court decisions, but they can apply four criteria to address traditional constitutional limits:

Sensor discretion is crucial. A nonintrusive detector that only discloses contraband has the best chance of being ruled permissible. Although Justice John Paul Stevens predicted that even the "perfectly discriminating mechanical sensor" would be prohibited by the *Kyllo* decision (*8*), the capability of the infrared camera most offensive to the Court was its potential to reveal lawful, intimate activities inside a home. Recently, a search dog sniffing outside a private residence was ruled admissible only because "…it did not explore the details of a house… and can do no more than reveal the presence or absence of contraband." (*9*). Portable mass spectrometers are being developed to detect and identify chemical and perhaps biological weapons. Would such a sensor that reveals all the volatile chemical substances in a residence be ruled acceptable?

To be truly effective, next-generation detection systems must be able to process all available signals—spectral, spatial, chemical, nuclear, and electromagnetic—but reveal no information except the presence or absence of contraband. Systems designed to support arms control and treaty verification include information barriers to meet similar requirements. Inspectors must confirm the presence of Special Nuclear Material in warheads being dismantled without revealing classified design information.

Performance must be well documented. Test data might be required to justify probable cause for a search. The issue of error rates will certainly surface. Justice David Souter's dissent in *Illinois* v. *Caballes* (in which the Court ruled that a dog sniff without articulable suspicion was permissible) stated that the decision in *United States* v. *Place* to allow dog sniffs was based on an untenable assumption "…that dogs do not err." (*10*). Designers must carefully characterize their systems to demonstrate overall effectiveness and specificity for contraband. If the systems have information barriers, then intermediate results that would normally confirm proper operation will not be available. Appropriate tests will have to be designed and used frequently.

Sensor deployment must be demonstrably effective. This is a matter for operations research and deterrence theory.

Sensors must be readily available. Unduly detaining or seizing an individual or belongings may be impermissible. Inexpensive, portable detectors along with widely networked communication systems can give law enforcement officers immediate access to information, enabling quick resolution of seizures.

If scientists and engineers understand the perspective taken by courts in the past, they will stand a much better chance of providing technical solutions that will balance the Fourth Amendment and civil liberties against the modern realities of terrorist threats.

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11. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-ENG-48. I am grateful to E. C. DuMont and D. J. Givelber for reading an earlier version.

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EVOLUTION

The Tree-Thinking Challenge

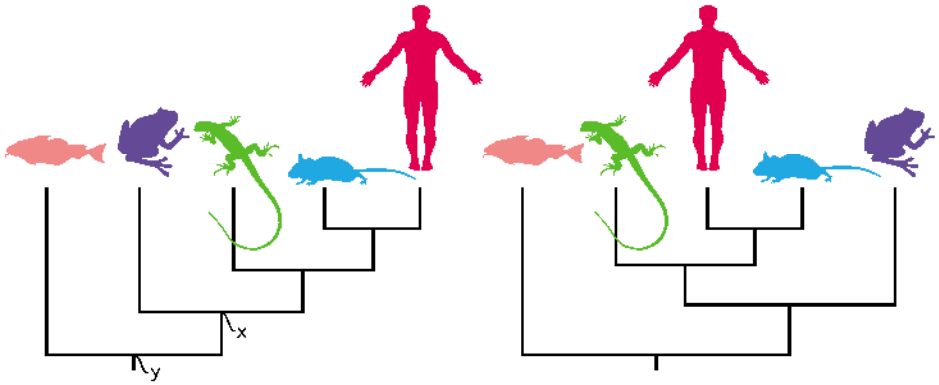
David A. Baum, Stacey DeWitt Smith, Samuel S. S. Donovan

The central claim of the theory of evolution as laid out in 1859 by Charles Darwin in *The Origin of Species* is that living species, despite their diversity in form and way of life, are the products of descent (with modification) from common ancestors. To communicate this idea, Darwin developed the metaphor of the "tree of life." In this comparison, living species trace backward in time to common ancestors in the same way that separate twigs on a tree trace back to the same major branches. Coincident with improved methods for uncovering evolutionary relationships, evolutionary trees, or phylogenies, have become an essential element of modern biology (*1*). Consider the case of HIV/AIDS, where phylogenies have been used to identify the source of the virus, to date the onset of the epidemic, to detect viral recombination, to track viral evolution within a patient, and to identify modes of potential transmission (*2*). Phylogenetic analysis was even used to solve a murder case involving HIV (*3*). Yet "tree thinking" remains widely practiced only by professional evolutionary biologists. This is a particular cause for concern at a time when the teaching of evolution is being challenged, because evolutionary trees serve not only as tools for biological researchers across disciplines but also as the main framework within which evidence for evolution is evaluated (*4*, *5*).

At the outset, it is important to clarify that tree thinking does not necessarily entail knowing how phylogenies are inferred by practicing systematists. Anyone who has looked into phylogenetics from outside the field of evolutionary biology knows that it is complex and rapidly changing, replete with a dense statistical literature, impassioned philosophical debates, and an abundance of highly technical computer programs. Fortunately, one can interpret trees and use them for organizing knowledge of biodiversity without knowing the details of phylogenetic inference. The reverse is, however, not true. One cannot really understand phylogenetics if one is not clear what an evolutionary tree is.

The preferred interpretation of a phylogenetic tree is as a depiction of lines of descent. That is, trees communicate the evolutionary relationships among elements, such as genes or species, that connect a sample of branch tips. Under this interpretation, the nodes (branching points) on a tree are taken to correspond to actual biological entities that existed in the past: ancestral populations or ancestral genes. However, tree diagrams are also used in many nonevolutionary contexts, which can cause confusion. For example, trees can depict the clustering of genes on the basis of their expression profiles from microarrays, or the clustering of ecological communities by species composition. The prevalence of such cluster diagrams may explain why phylogenetic trees are often misinterpreted as depictions of the similarity among the branch tips. Phylogenetic trees show historical relationships, not similarities. Although closely related species tend to be similar to one another, this is not necessarily the case if the rate of evolution is not uniform; Crocodiles are more closely related to birds than they are to lizards, even though crocodiles are indisputably more similar in external appearance to lizards.

But what does it mean to be "more closely related"? Relatedness should be understood in terms of common ancestry—the more recently species share a common ancestor, the more closely related they are. This can be seen by reference to pedigrees: You are more closely related to your first cousin than to your second cousin because your last common ancestor with your first cousin lived two generations ago (grandparents), whereas your last common ancestor with your second cousin lived three generations ago (great-grandparents). Nonetheless, many introductory students and even professionals do not find it easy to read a tree diagram as a depiction of evolutionary relationships. For example, when presented with a particular phylogenetic tree (see the figure, left), people often erroneously conclude that a frog is more closely related to a fish than to a human. A frog is actually more closely related to a human than to a fish because the last common ancestor of a frog and a human (see the figure, label x) is a descendant of the last common ancestor of a frog and a fish (see the figure, label y), and thus lived more recently. [To evaluate your tree-thinking skills, take the quizzes (*6*)].



Which phylogenetic tree is accurate? On the basis of the tree on the left, is the frog more closely related to the fish or the human? Does the tree on the right change your mind? See the text for how the common ancestors (x and y) indicate relatedness.

Why are trees liable to misinterpretation? Some evolutionary biologists have proposed that nonspecialists are prone to read trees along the tips (*1*, *7*), which in this case yields an ordered sequence from fish to frogs and ultimately to humans. This incorrect way to read a phylogeny may explain the widely held but erroneous view that evolution is a linear progression from primitive to advanced species (*8*), even though a moment's reflection will reveal that a living frog cannot be the ancestor of

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a living human. The correct way to read a tree is as a set of hierarchically nested groups, known as clades. In this example, there are three meaningful clades: human-mouse, human-mouse-lizard, and human-mouse-lizard-frog. The difference between reading branch tips and reading clades becomes apparent if the branches are rotated so that the tip order is changed (see the figure, right). Although the order across the branch tips is different, the branching pattern of evolutionary descent and clade composition is identical. A focus on clade structure helps to emphasize that there is no single, linear narrative of evolutionary progress (*1*, *7*).

There are other problems in reading relationships from trees (*9*). For example, there is a common assumption that trait evolution happens only at nodes. But nodes simply represent places where populations became genetically isolated, permitting them to accumulate differences in their subsequent evolution. Similarly, living species may be mistakenly projected backward to occupy internal nodes of a tree. But it is incorrect to read a tree as saying that humans descended from mice when all that is implied is that humans and mice shared a common ancestor. Thus, for all its importance, tree thinking is fraught with challenges.

Tree thinking belongs alongside natural selection as a major theme in evolution training. Further, trees could be used throughout biological training as an efficient way to present information on the distribution of traits among species. To this end, what is needed are more resources: computer programs (*10*), educational strategies (*11*, *12*), and accessible presentations of current phylogenetic knowledge (*13*–*15*).

Phylogenetic trees are the most direct representation of the principle of common ancestry—the very core of evolutionary theory—and thus they must find a more prominent place in the general public's understanding of evolution. As philosopher of science Robert O'Hara (*16*) stated, "just as beginning students in geography need to be taught how to read maps, so beginning students in biology should be taught how to read trees and to understand what trees communicate." Among other benefits, as the concept of tree thinking becomes better understood by those in the sciences, we can hope that a wider segment of society will come to appreciate the overwhelming evidence for common ancestry and the scientific rigor of evolutionary biology.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5750/979/DC1
Tree-Thinking Quizzes I and II

10.1126/science.1117727

CHEMISTRY

Following the Flow of Energy in Biomolecules

Paul M. Champion

Some biological molecules, such as those in visual or photosynthetic systems, have evolved to efficiently convert energy from one form to another. How do these molecules channel energy rapidly and efficiently so that useful work can be performed without this energy being dissipated ineffectively into the surroundings? Dissipation of molecular vibrational excitation energy typically takes place on picosecond time scales, so biological molecules must be able to channel energy rapidly and efficiently if they are to be able to direct it in a useful manner. In biological systems excited by light, the nonradiative electronic transitions can occur on time scales ($<< 10^{-12}$ ps) that are even faster than vibrational energy dissipation (*1*–*3*), hinting at how nature solves the problem of directing energy flow. On page 1006 of this issue, Kukura *et al.* (*4*) take an important step forward in defining the process of directed energy flow in the visual pigment rhodopsin.

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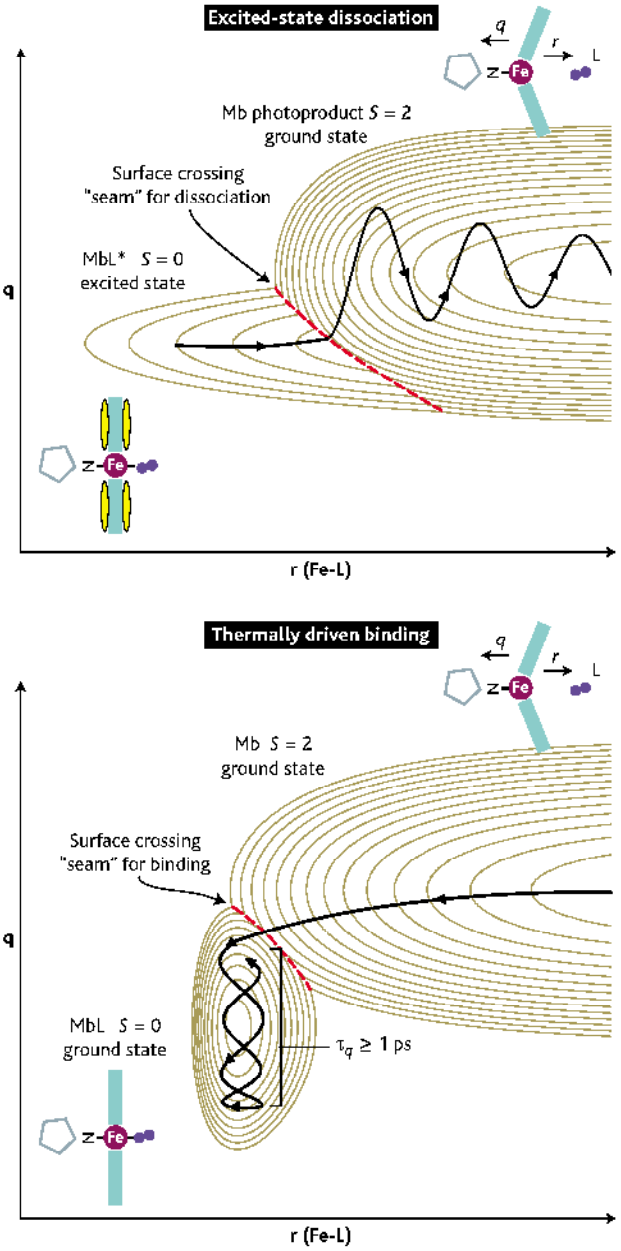
Photoexcited biological molecules offer a unique opportunity to monitor the evolution of excitation energy as it transforms a reactant molecule into its final products. With the advent of appropriate femtosecond laser techniques (*5*), it has become possible to examine the underlying dynamics of the elementary vibrational and electronic excitations that guide the structural changes and, ultimately, the function of a variety of biomolecules (*6*–*8*). The work presented by Kukura *et al.* enhances our ability to monitor rapid structural changes in such molecules by introducing the technique of femtosecond stimulated Raman spectroscopy (FSRS). In their report, Kukura *et al.* follow the evolution of the retinal chromophore as it is excited to photorhodopsin and decays into bathorhodopsin, all within the first picosecond of the visual process. They do this by taking advantage of the broad spectral bandwidth of their probe pulse to obtain very high quality time-resolved stimulated Raman spectra over the range of 600 to 2000 cm^{-1}.

How does this experiment generate ultrafast time resolution, as well as the high spectral resolution associated with Raman spectra, without violating the uncertainty principle? Although not emphasized in the report by Kukura *et al.*, these authors are fully aware (*9*) that the underlying time scale for the generation of the Raman photon is dictated by the dephasing time of the coherence between the initial and final vibrational levels of the material undergoing the Raman process. A typical time scale for the vibrational dephasing time is on the order of 10^{-12} s, which translates to a 10 cm^{-1} Raman bandwidth. This means that the FSRS experiment reads out Raman radiation from the sample that is averaged over its vibrational dephasing time window (that is, the stimulated Raman signals continue to appear at the detector, even after the probe pulse has passed through the sample). Thus, there is no violation of the uncertainty principle. However, being able to control the "gating" of the Raman coherence by changing the time delay between the photochemical pump and the broadband probe allows the dephasing time window to be moved so that rapid structural dynamics can be monitored. Changes in the vibrational frequencies that take place within the dephasing time window affect the FSRS lineshape, and the authors have done a convincing job of simulating these lineshape changes as shown in the supporting online material of their paper.

A key conclusion of the work on rhodopsin is that low-symmetry hydrogen out-of-plane (HOOP) wagging motions

allow the system to evolve extremely rapidly (~200 fs) onto the ground-state electronic surface of the product, where much of the ensuing structural change of the retinal chromophore (the cis to trans isomerization) actually takes place. This is a paradigm shift from the usual description of retinal isomerization reactions, where the electronic and nuclear structures are often taken to evolve together in time within a one-dimensional reaction coordinate model that involves multiple intermediate states. In contrast, a very rapid transition to the electronic ground state of the product leads to an impulsive nuclear response composed of those vibrational motions that are coupled to the electronic state changes (those nuclei that feel forces due to the change in the electron distribution are said to be "coupled"). Because these electronic forces are associated with the product electron distribution, they naturally direct the nuclei toward the final product structure with high efficiency following the rapid electronic transition. Intermediates on such a pathway are simply a measure of the progress of the structural part of the reaction on the product ground-state electronic surface.

Similar conclusions have been reached in femtosecond coherence studies of diatomic ligand dissociation from heme proteins (*7*, *10*). These studies show that it is the ultrafast transitions of the iron electrons that trigger and direct the resulting nuclear motion of the heme on its ground-state photoproduct electronic surface. The surface crossing "seam" for dissociation, shown in the top panel of the figure, is where the electronic part of the reaction takes place. The crossing seam is analogous to the conical intersection (*11*) mentioned by Kukura *et al.*, where HOOP modes couple the ground- and excited-state electronic surfaces. It remains unclear precisely what mediates the highly efficient coupling between the electronic surfaces in the heme system, but spin-orbit coupling, as well as coupling by out-of-plane heme-ligand stretching and bending modes, are likely candidates.



Good vibrations. (**Top**) A top-down view of the intersection of the initial photoexcited-state electronic surface (thin tan contour lines) of the heme in ligated myoglobin (labeled MbL*) and the photoproduct ground-state electronic surface (labeled Mb). After photoexcitation of the π-electrons of the heme chromophore (yellow region), the iron d-electrons rapidly reconfigure within their localized orbitals and go from a spin of $S = 0$ in MbL* to $S = 2$ in Mb. This exerts strong local forces on the nuclei surrounding the iron atom that move the system along the coordinate(s) q. The simplified picture depicts the photodissociation of the diatomic ligand (blue circles labeled L) along the iron-ligand coordinate r, as well as the coupling of the reaction to other chromophore and/or protein modes labeled q. The ensuing coherent vibrations of the reaction-coupled q-modes are specific to the ground state and appear within 100 fs of the photochemical pump (*7*, *10*). (**Bottom**) The thermally activated reverse reaction as the ligand binds to the heme along the ground-state electronic surface. The diatomic ligand is "trapped" by electronic coupling to nuclear coordinate(s) q when the period (τ_q) for the return to the binding seam is longer than the time it takes to dissipate vibrational energy.

Electron-nuclear coupling also plays an important role in thermally driven ground-state reactions (see the bottom panel of the figure, left). After the system accumulates enough thermal energy to surmount the energy barrier at the crossing seam, the forces of the electronic state change will guide the nuclear motion along q. When the vibrational period (τ_q) of mode q is longer than ~1 ps, the system loses enough vibrational energy before returning back to the crossing seam that it becomes trapped in the bound state. Without the reaction coupling to q, the system would rapidly (within the ~60-fs iron-ligand vibrational period) return to the crossing seam along r with enough energy to escape from the bound-state region. The electron-nuclear coupling of q gives the biological system the time it needs to dissipate energy within the bound-state region so that the efficiency of the binding reaction is optimized.

As a result of these studies, a scenario for directed energy transport is emerging in which biomolecules have evolved to make use of the fact that electrons are light and fast, whereas nuclei are heavy and slow. For ground-state reactions, the modes triggered by the electronic forces can help to trap the system in the desired electronic state. For photoexcited states, the fast electronic decay (mediated by motion along specific modes of appropriate symmetry and frequency) takes place before excess energy can escape to the surroundings, and this triggers highly specific electronic forces on the nearby nuclei when the electrons change state. Probably there is a correlation between the localization of the electronic state change and the specificity and efficiency of the nuclear (that is, structural) response in the associated reaction. In the event that the electronic transition is more delocalized, and therefore structurally less specific, the surrounding protein conformation may be called upon to act as a restraining lattice that helps to direct the elec-

tronic forces so that the structural part of the chromophore reaction is guided to the desired outcome. In turn, this can set up action-reaction forces on the protein that lead to specific and desired conformational changes extending over the much longer length and time scales necessary for the proper function of larger biological assemblies.

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BIOCHEMISTRY

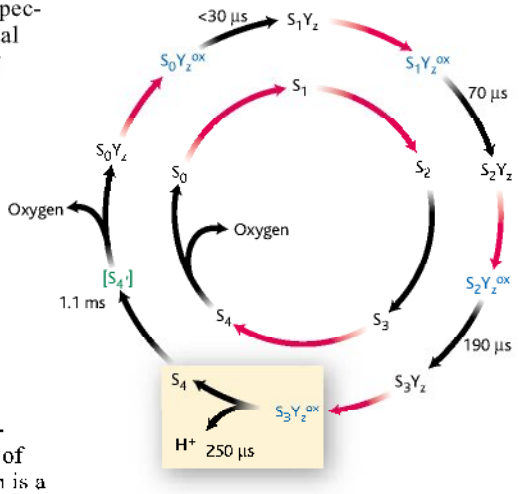
The Photosynthesis "Oxygen Clock" Gets a New Number

James E. Penner-Hahn and Charles F. Yocum

Despite decades of engineering effort devoted to solar energy conversion, artificial solar systems still capture only a trivial amount of energy compared with the amount captured by plants, green algae, and cyanobacteria through photosynthesis. On page 1019 of this issue, Haumann *et al.* (*1*) provide new insight into the mechanism of biological solar energy conversion. Using time-resolved spectroscopy to analyze the dynamical processes of photosystem II, they identify an important intermediate step in oxygen evolution. More generally, this demonstration that time-resolved structural data can be measured for the metal site in a dilute enzyme on time scales as short as 10 μs opens the door to more detailed characterization of biochemical kinetics of other metalloenzymes.

Photosynthesis converts solar energy into chemical energy with nearly 100% efficiency and negligible toxic by-products. At the heart of photosynthetic energy transduction is a multipolypeptide complex called photosystem II, which catalyzes the oxidation of water, splitting it into electrons and oxygen. The former product is used in the dark-reactions of photosynthesis to reduce carbon dioxide to the carbohydrates. This ultimately supplies food that is consumed by the rest of the biosphere. The latter product is the source of Earth's oxygen-rich atmosphere.

The catalytic center of photosystem II is the oxygen-evolving complex (also known as the water-oxidizing complex), a Mn_4Ca cluster that is the site of oxygen-oxygen bond formation. This is the rate-limiting chemical step in water oxidation. Recently, several crystal structures have begun to elucidate the structural organization of photosystem II, suggesting possible arrangements of the Mn and Ca ions (*2*–*4*). However, because of the low resolution of these data [positional uncertainties (*5*) ~1 to 1.5 Å] and the sensitivity of the crystals to radiation damage (*6*), detailed mechanistic questions regarding the chemistry of oxygen formation have had to rely on spectroscopic measurements. (Spectroscopy, unlike most crystallography, allows the system to be followed as it goes through its catalytic paces.) Almost all of the available wavelengths of light, from infrared to microwave and visible to x-ray, have been used to study photosystem II. Although each has provided a piece of information about the steps in the catalytic cycle of the oxygen-evolving complex, only microwaves (electron paramagnetic resonance) and x-rays (x-ray absorption spectroscopy) have been used successfully as specific probes of the catalytic site, and only x-ray absorption spectroscopy can be used to study each of the different oxidation states.



Mechanism of photosynthetic oxygen evolution. (Inner circle) Classical Kok cycle, showing five kinetically resolvable S states (S) of the manganese cluster of the oxygenic photosynthetic photosystem II reaction center. Red arrows indicate light-driven oxidation steps and black arrows indicate chemical steps. **(Outer circle)** Modern description of the Kok cycle, distinguishing between light-driven oxidation of a tyrosine cofactor by chlorophyll (red arrows) and kinetically resolvable chemical oxidations (black arrows). The rate constants of each chemical oxidation step (*1*) show that the x-ray absorption "edge" energy for the S_4 state (yellow box) is the same as that for S_3, suggesting that the Mn oxidation state is the same in S_3 and S_4. The putative S_4' state (green) may exist as a discrete species, or may simply represent a transition state between S_4 and the generation of the S_0 state and oxygen.

The basic mechanism of photosynthetic water oxidation has been known for nearly 40 years, since the discovery that oxygen is evolved after every fourth flash of light (*7*). This has implied that there must be at least five different states of the complex that are converted cyclically, known as the classical Kok cycle (*8*) (see the figure, inner circle). The five states are named S_0 to S_4, with the subscript indicating the number of oxidizing equivalents that are stored in the entire oxygen-evolving complex. It has since been recognized that this advancement of S states involves initial oxidation of a chlorophyll dimer (P_{680}), which in turn oxidizes a tyrosine cofactor, Y_Z, that is adjacent to the manganese cluster. In this model (see the figure, outer circle), the S-state nomenclature now refers specifically to the manganese oxidation state, and the tyrosine is denoted as Y_Z or Y_Z^{ox}.

The oxygen-evolving complex can be converted exclusively to the S_1 state by storage in the dark, and the S_0, S_2, and S_3 states can be trapped in high yield through various physical and chemical manipulations followed by rapid freezing. However, until recently the S_4 state has proven refractory, and this has limited efforts to understand the details of water oxidation. In par-

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ticular, it was unclear whether "S_4" existed as a discrete chemical intermediate, or whether it might simply represent a transition state containing S_3 with an oxidized tyrosine ($S_3Y_Z^{ox}$). The latter would imply an intimate role for Y_Z^{ox} in water oxidation, perhaps through hydrogen-atom transfer (*9*). In contrast, if S_4 exists as a discrete intermediate, then a range of mechanisms for the terminal reaction preceding oxygen formation are possible. A subtle delay in oxygen release relative to Y_Z^{ox} reduction (*10*, *11*) hinted that S_4 might exist as a discrete intermediate rather than simply being a transition state between S_3 and S_0. This conclusion was strengthened by a recent experiment showing that if one increases the partial pressure of oxygen on photosystem II, water oxidation is blocked at S_3. This suggests that increasing oxygen concentration shifts the equilibrium from S_4 oxygen to S_3 (*12*).

Haumann *et al.* (*1*) used a conceptually straightforward but experimentally challenging "pump-probe" time-resolved x-ray spectroscopy experiment to obtain direct structural evidence for an S_4 state. To appreciate the difficulty of this approach, it is important to remember that even "simple" static x-ray absorption spectroscopy of photosystem II is challenging because of the intrinsically low Mn concentration. The present measurements would have been impossible without the high-brightness third-generation synchrotron sources that provide higher x-ray flux. Kinetic traces (*1*) show clearly that the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ steps have very similar transient behavior, although the latter is somewhat slower. This finding is important because of the continuing controversy over whether Mn has been oxidized during the $S_2 \rightarrow S_3$ transition (*13*). The Haumann *et al.* data provide further support for the growing consensus that Mn is oxidized during both the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions. In contrast, the kinetic transient for the $S_3 \rightarrow \rightarrow S_0$ transition is distinct, with a 250-μs lag phase followed by a slow 1.1-ms transient phase. The latter phase is of opposite sign, representing Mn reduction to the S_0 state, and corresponds to the observed rate of oxygen release and reduction of Y_Z^{ox}. The former, more rapid phase provides direct evidence for the existence of a discrete S_4 intermediate state.

The lag phase indicates that the S_3 and S_4 states have similar x-ray absorption spectra and rules out several possible mechanisms for oxygen evolution. There has been widespread speculation that water oxidation might use a manganyl (Mn=O) species as the oxidant (*9*). This possibility was recently ruled out for S_3 (*14*). The present work by Haumann *et al.* extends this exclusion to S_4 because neither the S_3 nor S_4 state shows an intense transition on the low-energy side of the x-ray absorption "edge" (this is the abrupt increase in x-ray absorption cross section that occurs when the x-ray energy matches the binding energy of the Mn 1s electron). Such "pre-edge" transitions are the spectroscopic signature of manganyl species (*14*). Alternatively, the high-pressure oxygen studies (*12*) were interpreted in terms of an S_2 state with an associated H_2O_2 molecule for "S_4." This too is now excluded, because the Mn would be reduced in this state, relative to the previous S_3 state.

Haumann *et al.* (*1*) favor a model in which "S_4" contains $S_3Y_Z^{ox}$. That is, the fourth oxidizing equivalent in the water oxidation cycle resides on the tyrosine cofactor. On the basis of the positive reaction entropy and the equilibrium isotope effect for S_4 formation, they suggest that the 250-μs lag phase represents the lifetime for proton release from an intermediate chemical species bound to the oxygen-evolving complex. Tests of this and more detailed mechanistic studies will await future experiments. For now, the availability of the intense x-ray beams available at third-generation synchrotron sources has permitted the detection of a new intermediate in the water oxidation reaction. With this demonstration of feasibility, a wide range of other applications of microsecond time-resolved x-ray absorption spectroscopy to chemically and biologically important reactions can now be imagined.

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PLANETARY SCIENCE

What Do We Need to Know to Land on the Moon Again?

Maria T. Zuber and Ian Garrick-Bethell

In July 1969, the Apollo 11 lunar excursion module *Eagle* descended toward the Sea of Tranquility with Neil Armstrong in command. At 300 m above the lunar surface, short on fuel and looking for a smooth area on which to land, Armstrong "did not like what he saw. A crater as big as a football field was just ahead, surrounded by a field of boulders, some as big as Volkswagens" (*1*). Despite the obstacles, *Eagle* touched down safely, delivering the first human beings to the surface of the Moon in one of humankind's greatest technological achievements. As the United States and other nations actively plan to return to the Moon, a renewed discussion of the scientific knowledge of the lunar surface that is needed for future landings is appropriate.

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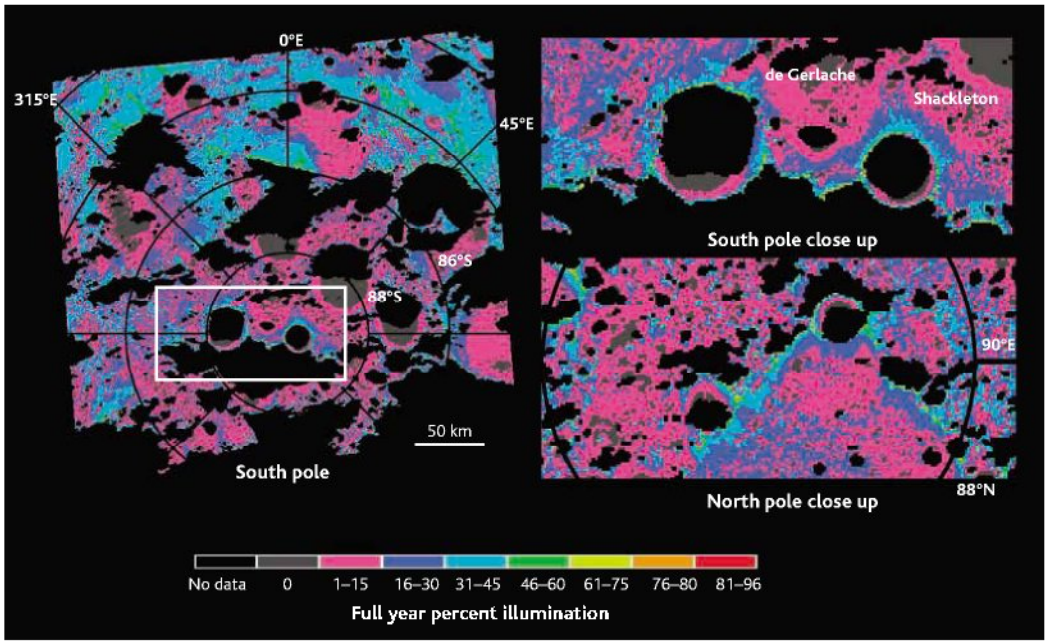
Of the dramatic and successful Apollo 11 landing, one thing can be said with certainty: We won't do it like that again. Starting with the Ranger 7 spacecraft and continuing with the Lunar Orbiters, images were used to characterize potential lunar landing sites by accumulating statistics of small-scale surface slopes and roughness. Most landings occurred in the maria, relatively smooth volcanic plains marred by small craters surrounded by rougher ejecta blankets and blocks. Two Apollo missions, 14 and 16, landed in non-mare (highland) regions, thanks to the skill of astronauts in manually piloting the lunar modules to locations safe enough for landing. But in today's risk-averse climate, the Apollo-era knowledge of the lunar surface—and, arguably, even our present knowledge—would not meet expectations with respect to safety. Future landings on the Moon, whether human or robotic, will demand a greater scientific knowledge of the lunar surface. In the selection of a landing site, two factors are relevant: landing safety and

fulfillment of mission objectives. Examples of the latter include in situ scientific hypothesis testing and resource assessment.

In the coming era of lunar exploration, a sensible and readily achievable modus operandi would be that future candidate landing sites undergo a level of scrutiny similar to that of the recent landed missions on Mars. The process to select the Pathfinder and Mars Exploration Rover landing sites (*2*, *3*) represents an extraordinarily successful example of how scientific information was used to make informed engineering decisions that in turn enabled scientific discovery. Whether the goal of a landed mission is driven by exploration or science (leaving aside esoteric debate concerning the difference between the two), the areas of greatest interest on the Moon will in general be more difficult to access and traverse than were the Apollo sites. In terms of scientific knowledge, a safe landing will require accurate characterization of local slopes on baselines of tens to hundreds of meters, and information about roughness on the scale of meters to decimeters (*4*). In addition, knowledge of soil properties combined with rock abundance and size distribution data will be required to assess "trafficability" of robotic rovers or human transport vehicles. On Mars, this knowledge has been achieved by careful analysis of candidate landing sites, using a combination of precise altimetry and high-resolution imaging at visible and thermal infrared wavelengths (*5*). These observations, coregistered with compositional information from orbital spectral sensors, led to the selection of the Meridiani Planum site that provided evidence of a water-rich past on Mars (*6*).

If we apply criteria used for landing site assessment at Mars to the Moon, our required knowledge is "not there yet" on a global basis. A primary order of business is being able to land precisely where one wants to go, which requires an accurate latitude-longitude grid referenced to the planetary center of mass. On the Moon, positional knowledge varies considerably with location. On the near side, limited locations are known relative to each other to within meters horizontally and to within centimeters radially, thanks to precise positioning provided by laser ranging to retroreflectors at Apollo sites and Soviet landers (*7*). But globally, absolute positions are known to no better than a few kilometers horizontally and 100 m radially (*8*). Positional knowledge on the far side is less well known than anywhere else on the Moon, in large part because of the poor quality of our knowledge of the lunar gravity field (*9*). In contrast, positions on Mars are known on a global basis to 100 m horizontally and 1 m radially (*10*). Without such knowledge on the Moon, precision landing is more complicated (*11*) and therefore riskier. The poor quality of the lunar geodetic grid would, for example, challenge our ability to explore the most topographically complex and scientifically important target on the far side: the South Pole–Aitken Basin, 2500 km in diameter and 8 km deep, a potential treasure trove for studying the internal composition of the Moon (*12*).

A key near-term goal of lunar exploration is resource assessment, in particular the definitive identification of water ice in permanently shadowed craters near the poles (*13*). Any long-duration mission in the vicinity of permanently shadowed craters would want to avail itself of another valuable resource: near-continuous sunlight that could satisfy power requirements (*14*). Unfortunately, topography of the quality needed to unambiguously determine constant darkness or illumination at all near-polar areas does not currently exist. As a case in point, the figure shows a full lunar year illumination cycle at both poles, using topography derived from Earth-based radar observations (*15*). The majority of south polar terrain is illuminated less than 50% of the time, although near two crater rims at the pole there is 4.7 km^2 of noncontiguous area illuminated more than 85% of the time, with a subset of this terrain receiving continuous light for more than 200 days per Earth year. In the north, however, there is only 1.1 km^2 of surface with more than 85% illumination, a discrepancy with illumination estimates obtained from Clementine spacecraft images collected over 71 days (*16*). Each data set has limitations; the radar suffers from nonoptimal viewing geometry and spatial resolution, whereas the Clementine images are limited by their short observation period. Thus, definitive conclusions concerning where best to land for missions with polar lighting constraints will require collection of a more complete data set.



Potential landing sites. (Left panel) Full lunar year illumination cycle at the south pole, calculated over 12 lunations (each 29.5 days) in 1994, from 10 January to 31 December, sampled every 4 hours. **(Top right)** Close-up of south polar region, with crater rims of de Gerlache and Shackleton dominating the highly illuminated terrain. **(Bottom right)** Same calculation for the north pole. Relative to the south pole, similar amounts of terrain are illuminated in the 1% to 60% range, but less area is found with higher illumination values.

Fortunately, help is on the way. Current and upcoming orbiters, notably ESA's Small Missions for Advanced Research in Technology (SMART-1, now in orbit), along with Japan's SELENE (2006), China's Chang'e 1 (2007), India's Chandrayaan-1 (2007), and NASA's Lunar Reconnaissance

Orbiter (2008), carry diverse payloads that will ensure that the fundamental geophysical, geological, and geochemical data needed to make informed decisions about where to land on the Moon will be available within the current decade. In the nearly 40 years since the Apollo 11 landing enthralled and inspired humankind, scientific information gained in the interim can guide and inform future missions, contributing to a rich and sustained program of lunar discovery.

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BIOMEDICINE

Separation of Conjoined Hormones Yields Appetite Rivals

Ruben Nogueiras and Matthias Tschöp

When we refer to our "gut feelings," not many of us actually visualize how the gastrointestinal tract spills myriads of small peptide hormones into our bloodstream to activate defined circuits of the central nervous system. Nevertheless, that picture does reflect a current scientific concept called the "gut-brain axis." This model consists of a complex network of hormonal and neuronal signaling pathways that is believed to balance numerous homeostatic and behavioral processes (*1*, *2*). In this context, our stomach does not just collect, process, and transport ingested food, but it also represents a multileveled conversational partner of the central nervous system. A key element of this communication process is the hunger-inducing hormone ghrelin, which is believed to convey information about nutrient availability from the stomach to the brain (*3*, *4*).

Zhang and colleagues (*5*) now report on page 996 of this issue that ghrelin not only has a sibling derived from the same peptide precursor (preproghrelin), but also that this new ghrelin-associated peptide behaves as a physiological opponent of ghrelin. Guided by bioinformatics-based predictions for typical enzymatic cleavage sites, they identified a 23–amino acid region of preproghrelin that is highly conserved across species, suggesting a relevant biological function. The authors purified a secreted peptide of the predicted size and sequence from rat stomach tissue and also detected it in rat blood. Similar to ghrelin, which requires posttranslational modification close to its amino terminus by acylation (*6*), the biological activity of the ghrelin-associated peptide also depends on modification, but by much more common amidation at its carboxyl terminus.

The surprising finding is the pharmacological effects of the newly identified peptide in comparison with the known actions of ghrelin. Whereas ghrelin increases food intake and body weight (*7*), the ghrelin-associated peptide decreases food intake and body weight gain in rodents. Moreover, Zhang *et al.* observed that the new peptide decelerates gastric emptying and decreases intestinal contractility in mice, both of which counteract the well-defined effects of ghrelin (*8*). Through a targeted screen of mammalian orphan receptors and subsequent analyses in cultured mammalian cells, Zhang *et al.* show that the ghrelin-associated peptide binds to and activates the orphan receptor GPR39 (*9*). This G protein–coupled receptor has been mapped to human chromosome 2 and is expressed in multiple tissues, including the stomach, intestine, and hypothalamus. This localization is consistent with a role in energy balance regulation (*10*). GPR39 is a member of a family that includes the receptors for ghrelin and motilin, another gastrointestinal hormone that stimulates food intake, gastric emptying, and gut motility (*9*, *11*). These facts support a somewhat counterintuitive, but nevertheless intriguing, relationship between ghrelin and the ghrelin-associated peptide.

To denote its anorexigenic actions, Zhang and colleagues named this new gastric hormone obestatin (from the Latin term *obedere*, meaning to "devour").

THE GHRELIN–MOTILIN RECEPTOR FAMILY MODULATES APPETITE AND GASTROINTESTINAL MOTILITY

Ligands	Receptors	Food intake	Gastric emptying
Motilin	Motilin-R (GPR38)	↑	↑
Neuromedin U	Neuromedin-R1 (GPR66), -R2	↓	↓
Neurotensin	Neurotensin-R1, -R2, -R3	↓	↓
Ghrelin	GHS-R	↑	↑
Obestatin	GPR39	↓	↓

The ghrelin-motilin receptor family and their ligands. Each of these gastrointestinal hormones acts on a specific G protein–coupled receptor from the same family to affect food intake and gastrointestinal motility (*9*–*11*). Similar dual effects on satiety and gastrointestinal motility are known for glucagon-like peptide 1, cholecystokinine, or peptide YY. Collectively, these peptides may serve to couple meal termination with inhibition of upper gastrointestinal function to prevent malabsorption and postprandial metabolic disturbances (*1*, *2*, *8*).

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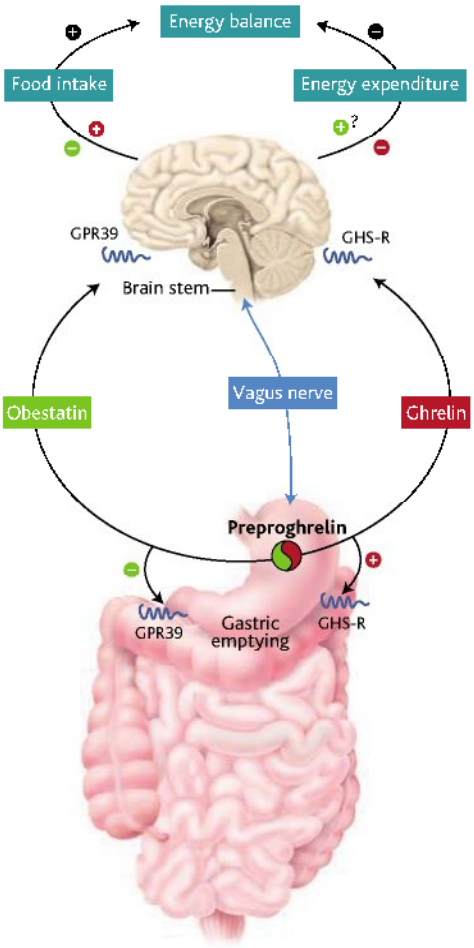
Inevitably, the terms "obesity" and "statins," a class of lipid-lowering drugs, come to mind. However, obestatin has not been tested in animal models of obesity and there is no evidence for a lipid-lowering effect. Furthermore, even its effect on body weight appears to be very subtle. The failure of obestatin treatment to decrease leptin levels in mice may indicate lack of lipolytic potency. Effects of obestatin on food intake regulation following administration to peripheral circulation or directly into the brain of mice suggest the typical action profile of a gastrointestinal satiety hormone. However, it is possible that obestatin may simply suppress appetite by triggering nausea or visceral illness. Recent examples have emphasized the importance of excluding nonspecific appetite suppression when examining anti-obesity drug candidates (*12*). Furthermore, despite sequence homologies between rodent and human obestatin (87%) and GPR39 (93%) sequences (*5*, *9*), data from rodents cannot always be translated to humans, where the effects of obestatin have yet to be determined.

Another concern regarding a role for obestatin in energy balance regulation arises from its quantification in blood. Although Zhang *et al.* confirmed earlier findings that the level of plasma ghrelin increases upon fasting and decreases following nutrient ingestion (*5*, *11*), they did not observe any changes in circulating obestatin upon fasting or feeding in rodents. Detection methods for differentiating between circulating amidated and nonamidated obestatin are not yet available, but could still reveal an association with nutrient availability. Nevertheless, total plasma obestatin generally appears to be a fraction of the level of plasma ghrelin. Should hormones derived from the same prepropeptide not circulate in an equimolar ratio?

Another peptide precursor that gives birth to antipodal regulators of food intake may provide some answers. The neuropeptide proopiomelanocortin is cleaved into several active fragments that include the appetite-suppressing α- and β melanocyte–stimulating hormones (α-, β-MSH) and the appetite-stimulating hormone β-endorphin (*13*). Tissue-specific enzymes determine which of these are generated. A similar scenario could determine how and where preproghrelin is fragmented into bioactive peptides. An earlier study postulated one other circulating preproghrelin fragment, a 13–amino acid peptide called C-ghrelin (*14*). In addition, turnover rates of ghrelin and obestatin may differ appreciably, according to their acylation or amidation rates, which again would be a parallel to the acetylation of the proopiomelanocortin derivative α-MSH (*15*). Dissecting the posttranslational cleavage, activation, or degradation processes of peptide hormones may reveal elegant enzymatic drug targets; Simultaneous activation of an agonist and deactivation of its endogenous functional antagonist could provide a powerful strategy for homeostatic control.

If obestatin lives up to its name as a circulating hormone with a physiologically relevant anorectic as well as an obesity-preventing function, the puzzling discrepancy between the very mild phenotype of mice lacking ghrelin (*16*, *17*) and the unsurpassed pharmacological effects of ghrelin on energy balance would receive an unexpected—but logical—explanation. The absence of an orexigenic hormone may be counterbalanced by the simultaneous deletion of an equally potent satiety factor. Targeted mouse mutagenesis is widely used as a strategy to unmask or validate the biological function of a gene product. An obvious abnormality of such a knockout mouse is usually interpreted as a reliable indicator of the target's physiological role. However, subtle or absent differences between gene-disrupted mice and their wild-type littermates are often regarded as evidence of negligible biological relevance. Such conclusions should be regarded with caution because developmental compensation may mask loss of function. However, rarely has such compensation been defined on a molecular level. The Zhang *et al.* findings caution against the interpretation of results based exclusively on gene disruption or messenger RNA quantification due to an additional level of complexity represented by posttranslational processing of proteins.

The discovery of obestatin leaves several questions unanswered. Why does a mouse that is deficient for the ghrelin receptor not exhibit an impressive phenotype? Should the absence of ghrelin action in the presence of an intact obestatin signaling pathway not generate a robust negative energy balance? Why does obestatin, unlike ghrelin, not affect growth hormone secretion from the pituitary gland, despite the presence of the obestatin receptor in this organ? Although the adversarial relationship between ghrelin and obestatin certainly is an important contribution to our understanding of body weight regulation, the search for a magic bullet against obesity is likely to continue—admittedly, a gut feeling.



The Yin and Yang personalities of ghrelin and obestatin. Both hormones derive from the same precursor protein and are predominantly secreted by the stomach and released into the blood. Each acts on a different receptor (GPR39 and GHS-R, as shown) and has an opposite effect on food intake, body weight, and gastrointestinal motility.

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Pattern-Oriented Modeling of Agent-Based Complex Systems: Lessons from Ecology

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Agent-based complex systems are dynamic networks of many interacting agents; examples include ecosystems, financial markets, and cities. The search for general principles underlying the internal organization of such systems often uses bottom-up simulation models such as cellular automata and agent-based models. No general framework for designing, testing, and analyzing bottom-up models has yet been established, but recent advances in ecological modeling have come together in a general strategy we call pattern-oriented modeling. This strategy provides a unifying framework for decoding the internal organization of agent-based complex systems and may lead toward unifying algorithmic theories of the relation between adaptive behavior and system complexity.

What makes James Bond an agent? He has a clear goal, he is autonomous in his decisions about achieving the goal, and he adapts these decisions to his rapidly changing situation. We are surrounded by such autonomous, adaptive agents: cells of the immune system, plants, citizens, stock market investors, businesses, etc. The agent-based complex systems (*1*) (ACSs) around us are made up of myriad interacting agents. One of the most important challenges confronting modern science is to understand and predict such systems. Bottom-up simulation modeling is one tool for doing so: We compile relevant information about entities at a lower level of the system (in "agent-based models," these are individual agents), formulate theories about their behavior, implement these theories in a computer simulation, and observe the emergence of system-level properties related to particular questions (*2*, *3*).

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Bottom-up models have been developed for many types of ACSs (*4*), but the identification of general principles underlying the organization of ACSs has been hampered by the lack of an explicit strategy for coping with the two main challenges of bottom-up modeling: complexity and uncertainty (*5*, *6*). Consequently, model structure often is chosen ad hoc, and the focus is often on how to represent agents without sufficient emphasis on analyzing and validating the applicability of models to real problems (*5*, *7*).

A strategy called pattern-oriented modeling (POM) attempts to make bottom-up modeling more rigorous and comprehensive (*6*, *8*–*10*). In POM, we explicitly follow the basic research program of science: the explanation of observed patterns (*11*). Patterns are defining characteristics of a system and often, therefore, indicators of essential underlying processes and structures. Patterns contain information on the internal organization of a system, but in a "coded" form. The purpose of POM is to "decode" this information (*10*).

The motivation for POM is that, for complex systems, a single pattern observed at a specific scale and hierarchical level is not sufficient to reduce uncertainty in model structure and parameters. This has long been known in science. For example, Chargaff's rule of DNA base pairing was not sufficient to decode the structure of DNA until combined with patterns from x-ray diffraction of DNA and from the tautomeric properties of the purine and pyrimidine bases (*12*). Thus, in POM, multiple patterns observed in real systems at different hierarchical levels and scales are used systematically to optimize model complexity and to reduce uncertainty.

POM was formulated in ecology, a science with a long tradition of bottom-up modeling. Ecology, in the past 30 years, has produced as many individual-based models as all other disciplines together have produced agent-based models (*13*), and has focused more on bottom-up models that address real systems and problems (*14*).

We describe here how observed patterns can be used to optimize model structure, test and contrast theories for agent behavior, and reduce parameter uncertainty. Finally, we discuss POM as a unifying framework for the science of agent-based complex systems in general.

Patterns for Model Structure: The Medawar Zone

Finding the optimal level of resolution in a bottom-up model's structure is a fundamental problem. If a model is too simple, it neglects essential mechanisms of the real system, limiting its potential to provide understanding and testable predictions regarding the problem it addresses. If a model is too complex, its analysis will be cumbersome and likely to get bogged down in detail. We need a way to find an optimal zone of model complexity, the "Medawar zone" (Fig. 1).

Modeling has to start with specific questions (*15*). From these questions, we first formulate a conceptual model that helps us decide which elements and processes of the real system to include or ignore. With complex systems, however, the question addressed by the model is not sufficient to locate the Medawar zone because ACSs include too many degrees of freedom. Moreover, the conceptual model may too much reflect our perspective as external observers, with our specific interests, beliefs, and scales of perception.

A key idea of POM is to use multiple patterns observed in real systems to guide design of model structure. Using observed patterns for model design directly ties the model's structure to the internal organization of the real system. We do so by asking: What observed patterns seem to characterize the system and its dynamics, and what variables and processes must be in the model so that these patterns could, in principle, emerge? For example, if there are patterns in age structure, sex ratio, and spatial distribution, then age, sex, and space should be represented in the

model; if we know that agents behave differently at high densities (e.g., are more aggressive), behavior variability should be in the model. This use of patterns might force us to include state variables and processes that are only indirectly linked to the ultimate purpose of the model and are not part of our initial conceptual model. Ideally, the patterns used to design a model occur at different spatial and temporal scales and different hierarchical levels, because the key to understanding complex systems often lies in understanding how processes on different scales and hierarchical levels are bound to each other.

Multiple patterns were key to modeling spatiotemporal dynamics of the beech forests of central Europe (Fig. 2). Natural beech forests are characterized by a spatial mosaic pattern of successional stages. A cellular automaton model that focused on this pattern only (*16*) was too poor in structure to reveal the forest's internal organization. But the forests have more characteristic patterns. Different successional stages have different patterns of vertical structure: e.g., the climax stage has closed canopy and little understory, and the decaying stage has canopy gaps and an understory of young beech. Therefore, a newer model (*17*, *18*) includes four height classes (from seedlings to upper canopy) (Fig. 2). The model also explicitly represents individual big trees because canopy gaps are caused by windthrow, an individual-level process. The model's structure was thus determined by the multiple characteristic patterns: The mosaic pattern determined horizontal spatial scale and resolution, the vertical patterns determined the need for height classes, and canopy gaps determined that large beeches must be described individually.

When designed to reproduce multiple patterns, models are more likely to be "structurally realistic" (*10*). In particular, model components (e.g., individuals) correspond directly to observed objects and variables, and processes correspond to the internal organization of the real system, so that the model "not only reproduces the observed real system behavior, but truly reflects the way in which the real system operates to produce this behavior" [(*19*), p. 5].

Structurally realistic models can make independent and testable secondary predictions. The beech forest model, for example, delivered independent predictions of forest characteristics that were not considered during model development and testing (*20*). Predictions of age structure in the canopy and the spatial distribution of very old "giant" trees were in good agreement with observations, considerably increasing the model's credibility and justifying a completely new application: tracking woody debris (*21*). Complexity in pattern-oriented bottom-up models is not simply a burden but can provide rich opportunities to increase model credibility, gain understanding (*18*), and address more questions.

In an example from ecological epidemiology, multiple patterns guided the stepwise design and calibration of a model describing the spread of rabies among red foxes in central Europe (*22*). Observed patterns included the large-scale wave of rabies prevalence, disease pockets ahead of the wave, and temporal oscillations of prevalence at local and regional scales. The resulting model reproduced these patterns, but not by simply applying a preconceived model structure and then fitting it to the patterns; instead, one pattern after another was used to gradually refine model structure (*23*). Structural realism of this model is indicated by the striking match between model predictions and a long-term data set of hunted foxes, which combines aspects of rabies epidemiology (before the onset of rabies control), fox ecology (after control), and their interaction (during control).

In other ACS disciplines, we found only a few models explicitly addressing multiple patterns, although many models were implicitly based on multiple patterns. A model of consumer markets (*24*) addresses three patterns: (i) The statistical distribution of weekly sales of fast-moving consumer goods has fatter tails and thinner peaks than normal distributions; (ii) there are clusters of high sales volatility; and (iii) market shares of different stores follow power-law distributions. Exactly how these patterns influenced the design of the model is not clear, but pattern (iii) appears to be why the model is spatially explicit: Consumer agents only visit stores that are nearby.

Patterns for Contrasting Alternative Theories

Agents continuously make decisions to reach their goals (e.g., survival and reproductive success, profiting in a stock market, finding the best place to settle) in an ever-changing environment. How do we model these decisions? What information do agents have, what alternatives do they consider, and how do they predict the consequences of their decisions? Many studies of ACSs try only one model of decision-making and attempt to show that it leads to results compatible with a limited data set. This practice, however, may lead to the impression that bottom-up models include so many parameters that they can be fitted to data whether or not their structure and processes are valid.

A more rigorous strategy for modeling agent decisions, or other bottom-up processes, is to use "strong inference" (*25*) by contrasting alternative decision models, or "theories" (*3*, *6*). First, alternative theories of the agent's decisions are formulated. Next, characteristic patterns at both the individual and higher levels are identified. The alternative theories are then implemented in a bottom-up model and tested by how well they reproduce the patterns. Decision models that fail to reproduce the characteristic patterns are rejected, and additional patterns with more falsifying power can be used to contrast successful alternatives. Rigorous techniques can be used to design experiments and analyze data (*6*, *26*).

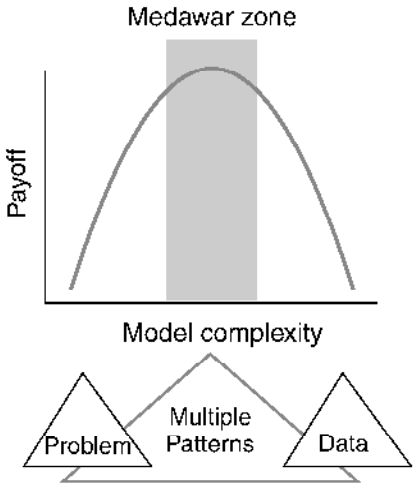

Fig. 1. Payoff of bottom-up models versus their complexity. A model's payoff is determined not only by how useful it is for the problem it was developed for, but also by its structural realism; i.e., its ability to produce independent predictions that match observations. If model design is guided only by the problem to be addressed (which often is the explanation of a single pattern), the model will be too simple. If model design is driven by all the data available, the model will be too complex. But there is a zone of intermediate complexity where the payoff is high. We call this the "Medawar zone" because Medawar described a similar relation between the difficulty of a scientific problem and its payoff (*41*). If the very process of model development is guided by multiple patterns observed at different scales and hierarchical levels, the model is likely to end up in the Medawar zone.

As an example, consider the well-known "boids" model (*27*) that produces schooling-like behavior from a simple theory: Individual boids try to avoid collisions, match the velocity of neighboring individuals, and stay close to neighbors. The emergence of aggregations resembling fish schools from this theory (Fig. 3), however, does not prove that boids explains schooling in real fish.

To define theory for schooling of real fish, Huth (*28*) used observed patterns and contrasted alternative theories for fish behavior. Two patterns characterizing fish schools were defined and quantified: polarization and nearest neighbor distance (Fig. 3). Eleven alternative theories for how fish adapt swimming speed and direction were formulated. In the first nine theories, the influence of neighbors is averaged; but in two theories, fish adjust their swimming to only one neighbor—e.g., the one

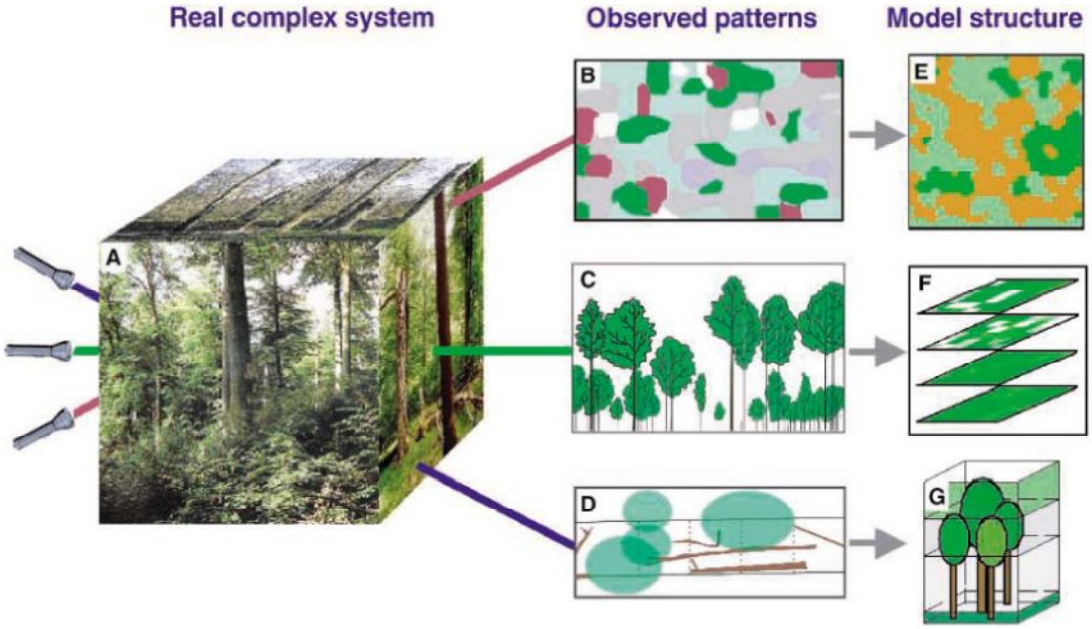

Fig. 2. Pattern-oriented model design. Observed patterns that characterize old-growth beech forests [(A); images: front, M. Flade; right, C. Rademacher; top, S. Winter] include a horizontal mosaic of developmental stages [(B); *x* scale: 400 m; modified from (*42*)], the vertical patterns of tree size that define the developmental stages [(C), showing the late decaying stage; *x* scale: ~60 m; modified from (*43*)], and distributions of fallen large trees [(D), a map of fallen wood; ellipses indicate crown projections of standing trees; *x* scale: ~60 m; modified from (*43*)]. To allow these patterns to emerge from it, the model includes a grid-based horizontal structure [(E), showing grid cells in three developmental stages; *x* scale: 570 m], a grid-based vertical structure [(F), showing each grid cell's percentage cover for four height classes; total area shown: 1 ha)], and individual representation of large trees [(G), showing one cell's trees in the largest two height classes; cell area: 204 m^2); (E) to (G) modified from (*18*)].

closest in front. These two "priority" theories failed to reproduce realistic polarization values (Fig. 3), eliminating them as valid theory.

This example shows that looking at one pattern may not be sufficient to falsify weak theory: Looking at nearest neighbor distance alone suggested that both types of schooling model produce similar results, but in fact the priority theories produce schools only as compact, but not as polarized, as real schools. Moreover, the nine theories based on averaging differ widely in assumptions, but the fish school's properties turned out to be robust to these assumptions. Demonstrating robustness is also key to a bottom-up model's credibility, because it indicates that we captured the most important mechanisms. Huth and Wissel's model also reproduced several additional patterns not considered during model development, providing further support for its structural realism.

This pattern-oriented theory development approach is increasingly used in models of ACS. Railsback and Harvey (*9*) used a stream trout model to contrast three theories for how individual fish select habitat. Only a new theory that assumes that fish select habitat to maximize expected survival over a future period reproduced observed patterns of feeding hierarchy, response to competing species and predatory fish, seasonal habitat shifts, and response to reduced food availability. Although these patterns are each qualitative, or "weak," together they were able to falsify all but one theory of habitat selection.

In a model exploring what determines the access of nomadic herdsmen to pasture lands owned by village farmers in north Cameroon, herdsmen negotiate with farmers for access to pastures (*29*). Two theories of the herdsmen's reasoning were contrasted: (i) "cost priority," in which herdsmen only consider one dimension of their relationship to farmers' costs; and (ii) "friend priority," in which herdsmen remember the number of agreements and refusals they received in previous negotiations. Real herdsmen sustain a social network across many villages through repeated interactions, a pattern reproduced only by the "friend priority" theory.

In economics, agent-based model experiments have been used to identify characteristics of artificial stock market investors that reproduce patterns well known from real stock markets (*30*). These patterns include continual and unpredictable stock price volatility, high skew and kurtosis in the distribution of profits among investors, and an inverse relation between current investment profits and future price instability. Two assumptions were contrasted about how much historic data investors use to predict the outcome of their investment decisions: (i) Investors all use 25-year memories of market data, versus (ii) memory varies from 0.5 to 25 years. Although none of the simulations reproduced all the observed market patterns, the assumption that all investors use 25-year memories failed to reproduce the most basic pattern: price volatility. This pattern-oriented analysis indicates that individual variation in investment decision-making is crucial to stock market dynamics.

Testing and contrasting alternative theories or decision models has several benefits. We are forced to be explicit about how decision models are formulated and tested; we can demonstrate how important the specific formulation of a decision or any other low-level—model is; we can explore null models; and we can continually refine models by applying additional patterns.

Patterns for Parameters: Coping with Uncertainty

Pattern-oriented modeling can reduce uncertainty in model parameters in two ways. First, it helps make models structurally realistic, which usually makes them less sensitive to parameter uncertainty (*31*). For example, an individual-based coyote population model reproduced an array of observed patterns with no fine-tuning of parameter values taken from the literature (*32*). The trout model (*9*) had four parameters that were particularly uncertain yet important; each had relatively independent effects on four different outputs (size versus abundance, for juveniles versus adults), so they could be calibrated manually and independently.

Second, the realism of structure and mechanism of pattern-oriented models helps parameters interact in ways similar to interactions of real mechanisms. It is therefore possible to fit all calibration parameters by finding values that reproduce multiple patterns simultaneously. This technique is known as "inverse modeling" (*33*). For a spatially explicit individual-based model of brown bear dispersal from Slovenia into the Alps (*34*), a global sensitivity analysis of the uncalibrated parameter set revealed high uncertainty in

model output. To reduce this uncertainty, two data sets were used to identify five patterns. Quantitative criteria for the agreement between observed and simulated patterns were developed. The indirect modeling analysis started with 557 random parameter sets covering the plausible ranges of all parameters. The five observed patterns were used as filters: Only 10 of the 557 parameter sets reproduced all of them. This parameter filtering reduced the model's global sensitivity by a factor of 4 (fig. S1).

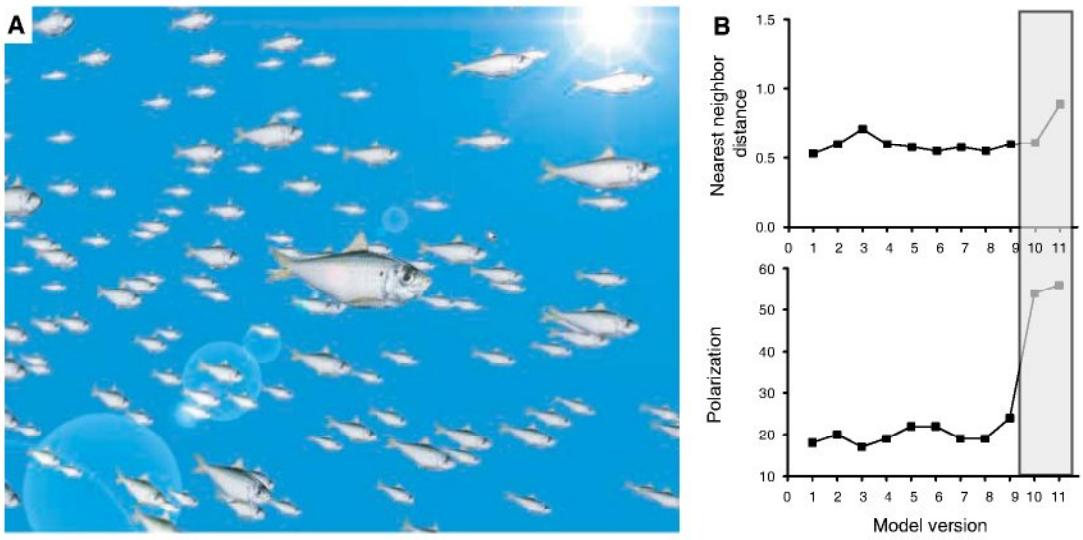

Fig. 3. Strong inference by contrasting alternative theories of the agents' behavior. Boids (27) is a conceptual model that demonstrates how schools or flocks can emerge from simple rules for behavior [(A); a version of boids by H. Hildenbrandt (*44*)]. (B) In a similar model of fish schools (*28*, *45*), 11 alternative theories of fish behavior were contrasted by looking at two school-level patterns: polarization (p) and nearest neighbor distance (*NND*); p is 0° if all fish swim in the same direction and p approaches 90° if all fish swim in random directions. Values of p observed in real fish schools are 10° to 20°; observed *NND* is often <1 fish body length. In model versions 1 to 9, the influence of neighbor fish is averaged; in model version 10 and 11 (shaded), fish select a single neighbor fish and orient their swimming to this neighbor only.

Indirect parameterization is routine in physical process models (i.e., in chemistry, hydrology, and climate modeling), but rare so far in models of ACSs. An encouraging exception is the agent-based model of an ancient society, the Kayenta Anasazi, who occupied the Long House Valley in northeastern Arizona (United States) until 1300 A.D. Paleoenvironmental and archaeological records permitted the development of a detailed, spatially explicit agent-based model of this society and its history (*35*). These data include estimates of annual potential maize production for each hectare in the study area for the period 400 to 1400 A.D. and records of human settlement in the valley. Theories for agent decisions, for example, splitting households and moving, were based on detailed regional ethnographies.

The model includes variability in mortality, fertility, splitting of households, and maize harvest rates; with eight unknown parameters. To evaluate these parameters indirectly, the time series of the number of simulated households was compared to the historical record. The best parameter set reproduced all important trends and population sizes in the archaeological record. This parameter set also reproduced important features of the spatial distribution of the settlements (Fig. 4) and the gradual northward movement of the population. These spatial patterns can be considered independent predictions, strong indicators of the model's structural realism.

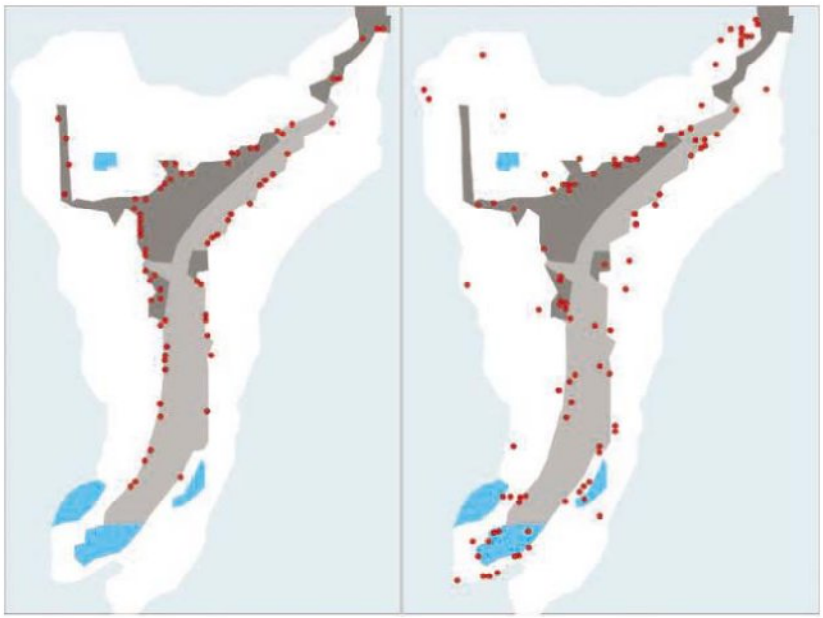

Fig. 4. Parameterization and independent predictions of an agent-based model of the Anasazi in the Long House Valley [modified from (*35*)]. The simulation environment consists of an 80 by 120 grid of 1-ha squares. Dark gray represents a higher water table; light gray and blue represent a lower water table. White is nonfarmable land. The red dots represent settlements. (Left) The historical settlement in 1125 A.D.; (right) prediction of the simulation model for the same year. The match between data and simulation is imperfect, but the clustering of settlements along the valley boundaries is captured by the model. The model was calibrated not to the settlement patterns but to the population size time series for 400 to 1450 A.D.

Implications and Future Directions

Patterns are widely used by many modelers, particularly in disciplines where the low-level entities are physical objects such as atoms and stars, or are relatively easy to represent, such as flocking birds, pedestrians in a panicking crowd, or car drivers ["Brownian agents" (*36*); see also table S1]. However, POM is the first attempt to explicitly formulate a rigorous and comprehensive strategy for modeling ACSs. The POM strategy is a

way to focus on the most essential information about a complex system's internal organization. Multiple patterns keep us from building models that are too simple in structure and mechanism, or too complex and uncertain. Using patterns to test and contrast alternative theories for agent behavior or other low-level processes is a way for the science of ACS to get beyond clever demonstration models and on to rigorous explanations of how real systems are organized and how they respond to internal and external forces. POM is just taking root, and we expect to see its rapid development in the near future.

Bottom-up models are virtual laboratories where controlled experiments distinguish noise from signal in the system's organization. In particular, experiments contrasting hypotheses for the behavior of interacting agents will lead to an accumulation of theory for how the dynamics of systems from molecules to ecosystems and economies emerge from bottom-level processes. This approach may change our whole notion of scientific theory, which until now has been based on the theories of physics. Theories of complex systems may never be reducible to simple analytical equations, but are more likely to be sets of conceptually simple mechanisms (e.g., Darwinian natural selection) that produce different dynamics and outcomes in different contexts. POM thus may lead us to an algorithmic (*37*), rather than analytical, approach to theory.

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11. Patterns are observations of any kind showing nonrandom structure and therefore containing information on the mechanisms from which they emerge. Complex systems contain patterns at different hierarchical levels and scales. Ecosystems, for example, contain patterns in primary production, species diversity, spatial structure, dynamics of component species populations, behavior of individual organisms, resource dynamics, and response of all these to disturbance events and stress. Useful patterns need not be striking; qualitative or "weak" patterns can be powerful in combination. For example, we can easily identify a person in a crowd even without a strong pattern (e.g., a photograph) by using a set of weak patterns: sex, approximate age, hair color, size, etc. Each of these characteristic patterns excludes many individuals.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/310/5750/987/DC1
SOM Text
Fig. S1
Table S1
References and Notes

10.1126/science.1116681

Multiple Transatlantic Introductions of the Western Corn Rootworm

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Prevention of biological invasions, as opposed to remedial eradication of invasive species, represents the most cost-effective and perhaps only hope for stemming the current homogenization of the world's biota (*1*). Here we describe the introduction routes into Europe of the western corn rootworm (*Diabrotica virgifera virgifera*, WCR), the most destructive pest of corn in the United States. Armed with this knowledge, it will be possible to better gauge the prevention strategies that might be adopted.

WCR was first detected in Europe in the former Yugoslavia in 1992 and has since spread throughout much of central and southeastern (CSE) Europe (*2*). Outbreaks of WCR were subsequently detected in northeast Italy in 1998 (in Veneto), 2002 (in Pordenone), and 2003 (in Udine); in northwest Italy and Switzerland in 2000; near Paris, France, in 2002 and 2004; and in eastern France, Switzerland, Belgium, the United Kingdom, and the Netherlands in 2003 (*2*). Although the invasion history of WCR is well documented, the source populations of the Western European outbreaks remain unknown. Because of the sequence of outbreaks, CSE Europe was generally assumed to be the source of most, if not all, the Western European populations (*3*). However, in principle, each outbreak could have originated from North America, CSE Europe, or one of the other Western European foci.

To discriminate between these introduction scenarios, we analyzed the genetic variation of European and American WCR populations at eight microsatellite loci (*4*, *5*). Simple genetic statistics gave useful but qualitative insights into the origin of most European outbreaks (*5*) (table S1). We then used a model-based approximate Bayesian computation (ABC) method relying on computer simulations (*5*, *6*) to quantitatively compare the different introduction scenarios for the Western European WCR populations (Fig. 1).

Our results are clear-cut and unexpected. Two of the Western European populations analyzed did not originate from CSE Europe but directly from North America; this scenario was supported by Bayes factors (BF) higher than 10^5 and posterior weights (PW) of ~1 for the northwestern Italy and Paris 2002 populations. Moreover, these introductions were independent from each other ($BF \geq 159$ and $PW \geq 0.94$). According to our analysis, the northeastern Italy 2003 outbreak was the only one to originate from CSE Europe ($BF = 183$ and $PW = 0.94$), and the eastern France population was derived from the Paris 2002 population ($BF = 3.9$ and $PW = 0.45$). The only population with ambiguous origins was Paris 2004, which could have been derived either from North America ($BF = 2.05$ and $PW = 0.70$) or from Paris 2002 ($PW = 0.22$). The presence of unsampled European populations acting as alternative introduction sources for the three primary outbreaks (CSE Europe, northwestern Italy, and Paris 2002) could be ruled out. This was true whether the unsampled population was one of those detected in 2003 ($BF > 10^4$ and $PW \sim 1$) or a hypothetical population founded in the 1980s ($BF > 3.6$ and $PW > 0.68$).

It has been widely assumed that the European WCR invasion was the result of a single unpredictable introduction. Our finding that there have been at least three independent transatlantic introductions of WCR suggests that incursions from North America are chronic. Prevention of future WCR invasions will require action against multiple invasion routes, which have apparently been used repeatedly and are potentially predictable. Our study also raises questions concerning the changing circumstances (such as adaptation by the insect or changes in control measures or transportation practices) that have permitted a sudden and recent burst of transatlantic introductions of WCR.

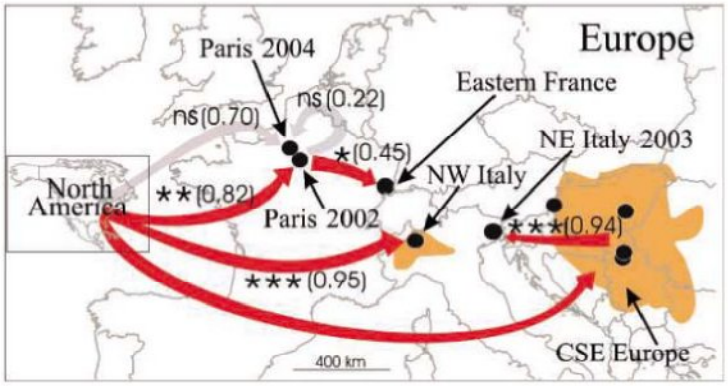

Fig. 1. The most likely scenarios of invasion into Europe by WCR, deduced from the ABC analysis. For each European outbreak, a red arrow indicates its most likely origin; the *PW* values of the introduction scenarios are in parentheses. Gray arrows represent unresolved scenarios. Large areas where WCR is present are shown in orange. *BF* values supporting the most likely scenarios of 3.2 to 10 (substantial support), 10 to 100 (strong support), and >100 (decisive support) are indicated by one, two, or three asterisks, respectively; ns, not supported.

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Supporting Online Material

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Materials and Methods
Table S1
References and Notes

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Transient Floral Change and Rapid Global Warming at the Paleocene-Eocene Boundary

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Rapid global warming of 5° to 10°C during the Paleocene-Eocene Thermal Maximum (PETM) coincided with major turnover in vertebrate faunas, but previous studies have found little floral change. Plant fossils discovered in Wyoming, United States, show that PETM floras were a mixture of native and migrant lineages and that plant range shifts were large and rapid (occurring within 10,000 years). Floral composition and leaf shape and size suggest that climate warmed by ~5°C during the PETM and that precipitation was low early in the event and increased later. Floral response to warming and/or increased atmospheric CO_2 during the PETM was comparable in rate and magnitude to that seen in postglacial floras and to the predicted effects of anthropogenic carbon release and climate change on future vegetation.

At the beginning of the Eocene Epoch ~55.8 million years ago, global temperatures increased by 5° to 10°C over a period of ~10 to 20 thousand years (ky) then returned to warm background climates over the succeeding ~100 ky (*1*–*4*). This event, the Paleocene-Eocene Thermal Maximum (PETM) (*5*), coincided with a global negative carbon isotope excursion (CIE) and calcium carbonate dissolution in the deep ocean, which are consistent with a large release of ^{13}C-depleted carbon to the ocean and atmosphere (*6*). Several sources have been proposed for this carbon: ocean-floor clathrates (*7*), thermogenic methane (*8*), and burning of peat and/or shallowly buried coals (*9*).

Biotic events at the PETM include mass extinction among benthic foraminifera (*10*), changes in the latitudinal range and species composition of marine plankton (*11*, *12*), and shifts in the taxonomic and trophic composition of terrestrial vertebrate faunas, probably after dispersal over high-latitude land bridges (*13*, *14*). Although the distribution and diversity of terrestrial plants are strongly influenced by climate today, previous work has shown little mega- or palynofloral change across the Paleocene-Eocene interval (*15*–*19*). Here we report terrestrial megafloras from the PETM and use them to infer change in the climate and floral composition in the interior of North America.

Geological framework. Our data come from the upper Fort Union and lower Willwood formations in the Cabin Fork drainage, southeastern Bighorn Basin, Wyoming, United States (~43.96°N, 107.65°W) (Fig. 1). These sediments were deposited by small fluvial systems near the margin of a subsiding intermontane basin, and they preserve a suite of environments including small channels, floodplain paleosols and swamps, and abandoned channel fills. We measured strata with a Jacob's staff and sighting level, then correlated sections by tracing beds with a differential Global Positioning System to create a stratigraphic and biostratigraphic framework with ~1-m resolution (Fig. 2).

Two lines of evidence establish the PETM age of these strata: mammalian biostratigraphy and $\delta^{13}C$ of paleosol organic matter. Fossil mammals indicating the late Paleocene Clarkforkian North American Land Mammal Age (NALMA) were found from 5 to 22 m below the top of the Fort Union formation (Fig. 2). The main fossiliferous layer is a laterally extensive, ferruginous, grit-pebble conglomerate that has produced >200 specimens and 11 species. The presence of *Copecion*, an abundance of *Phenacodus* and *Ectocion*, and the absence of *Hyracotherium* indicate that this fauna belongs to the latest Clarkforkian zone Cf-3 (*20*, *21*). The earliest Eocene mammals (Wasatchian NALMA, the Wa-0 zone), which occur within the CIE in other areas (*19*, *21*–*25*), come from the lowest 37 m of the Willwood formation. Nineteen species are represented among 233 specimens, including diagnostic Wa-0 taxa (*Arfia junnei*, *Copecion davisi*, *Hyracotherium sandrae*, and *Diacodexis ilicis*) (*25*). The lowest Wa-0 fossils come from paleosols and clay clast accumulations in sandstones 3 to 5 m above the base of the Willwood formation and 8 m above the highest Clarkforkian mammals. The highest Wa-0 fossils occur 37 m above the base of the Willwood formation and 3 m below three thick, laterally persistent, red paleosols. In the Cabin Fork area, the highest of these three persistent paleosols (at 47 m) produced 10 species of mammals, including *Cardiolophus radinskyi*, which defines the succeeding Wa-1 faunal zone (*25*) (Fig. 2). Thus, the Wa-0 faunal zone in the Cabin Fork area is at least 34 m thick and is bounded by Cf-3 and Wa-1 faunas.

We measured the carbon isotopic composition of bulk organic matter ($\delta^{13}C_{org}$) from mud-rock paleosols in the same sections (*26*) (Fig. 2A and fig. S1). $\delta^{13}C_{org}$ ranged from −22 to −28.5 per mil (‰) and, when grouped into PETM and non-PETM samples based on faunal criteria, was strongly negatively correlated with the weight percent of organic carbon (wt % C_{org}), which varies from 3.6% to 0.05% (fig. S1 and table S1). We plotted deviations of $\delta^{13}C_{org}$ from the values expected based on wt % C_{org} (*26*) (Fig. 2A and fig. S1). The carbon isotope curve shows a sharp excursion of −3.3‰, starting 2 to 3 m below the lowest occurrence of Wa-0 mammals. The magnitude of the CIE is similar to that in soil organic matter at Polecat Bench in the northern Bighorn Basin (*27*) (Fig. 2B). Our isotope anomaly values remain 2 to 3‰ below background values throughout the 50 m of section above the base of the CIE, with the exception of a single more positive sample at 5 m (Fig. 2A) that was poorly consolidated and contaminated with modern roots. The lowest Wa-1 mammals occur within the upper part of the CIE, as is seen at Polecat Bench (*27*) (Fig. 2B).

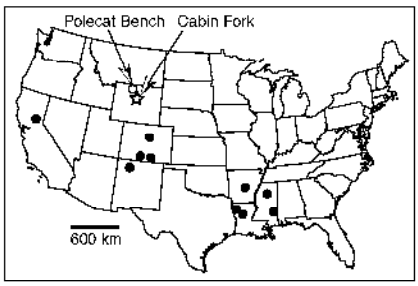

Fig. 1. The location of the Cabin Fork and Polecat Bench PETM sections. Solid dots indicate Paleocene and Eocene sites with plant types that are restricted to the PETM in northern Wyoming.

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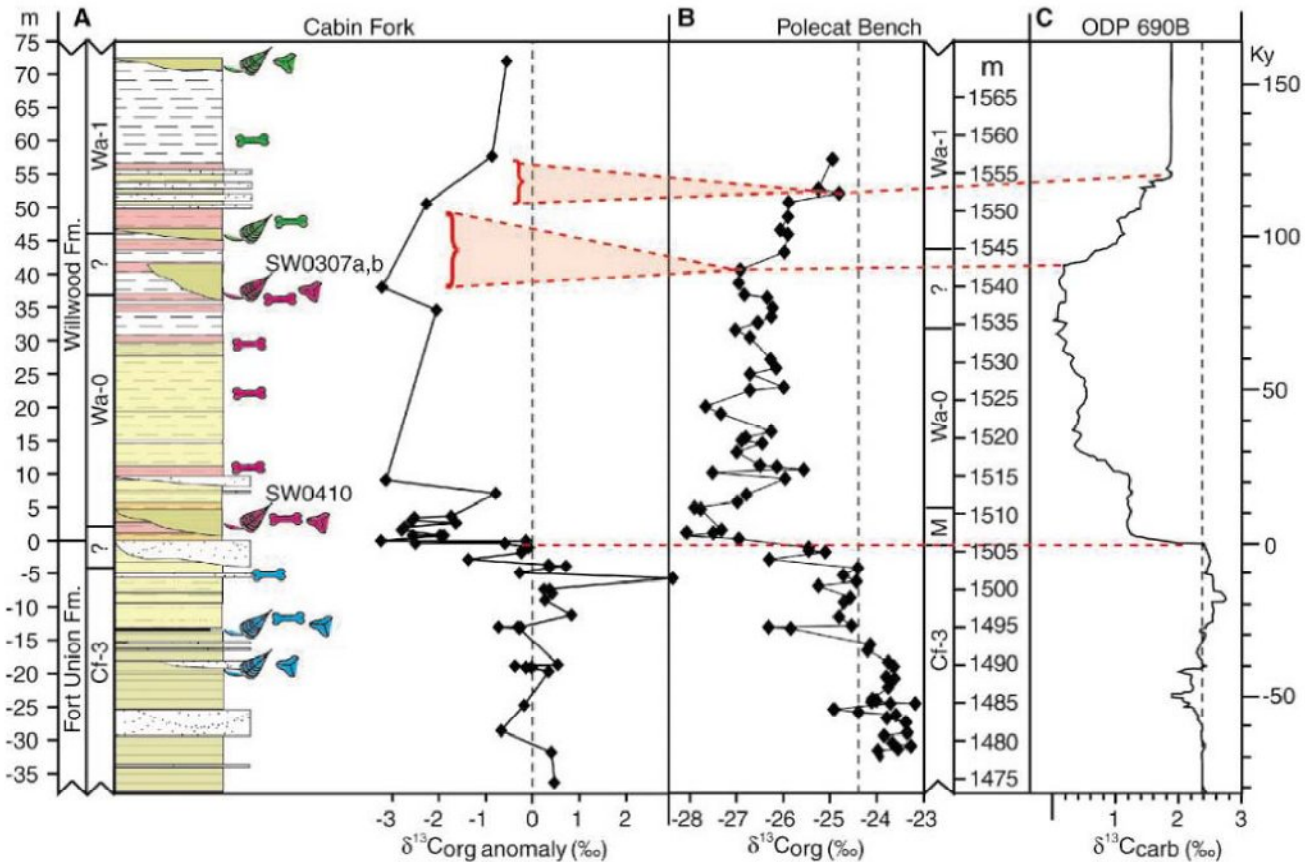

Fig. 2. Comparison of PETM records. (**A**) The Cabin Fork section, showing meter levels, formations, faunal zones, lithology, fossil sites, and $\delta^{13}C_{org}$ anomaly values (*26*). (**B**) The Polecat Bench section, showing $\delta^{13}C_{org}$, faunal zones, and meter levels (*27*). (**C**) $\delta^{13}C$ of bulk carbonate at Ocean Drilling Program (ODP) site 690B (in the Southern Ocean), with the time scale from Farley and Eltgroth (*3*). Wa, Wasatchian; Cf, Clarkforkian; M, *Meniscotherium* Zone. Paleocene fossils are indicated with blue symbols, PETM with red, and post-PETM Eocene with green. Carbon isotope units are in ‰ Pee Dee belemnite. Dashed orange lines indicate correlations of carbon isotope curves. Dashed vertical lines are mean $\delta^{13}C$ vales for the latest Paleocene.

Floral composition and migration. Plant fossils were collected from lenticular channel fills 3 to 5 m thick and <50 m across. Because of small-scale downcutting and redeposition, plant fossils are slightly younger than overbank deposits at the same level; however, the continuous floodplain paleosols above the channel fills are within the PETM, as indicated by vertebrate fossils and/or $\delta^{13}C_{org}$ anomaly values.

Two localities, SW0410 and SW0307 (3 and 37 m above the base of the CIE, respectively), produced a total of 398 plant megafossil specimens [136 and 262, respectively (table S3)]. The lower locality has nine leaf morphospecies, including six dicots, one palm, and one fern. The upper locality has 20 leaf morphospecies, including 17 dicots, one palm, and two ferns. In composition, both PETM megafloral localities are dominated by morphospecies that have not been recognized in extensive collections (~30,000 specimens from >300 localities) from the late Paleocene and early Eocene of the Bighorn Basin (Fig. 3) (*28*).

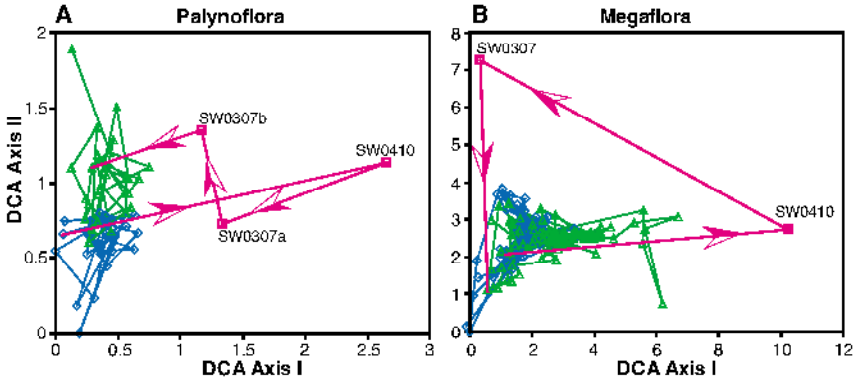

Fig. 3. Change in floral composition analyzed with detrended correspondence analysis (DCA). Each bivariate plot was generated by DCA of a sites-by-species matrix of presence/absence data. Arrows indicate the temporal sequence. (**A**) Palynofloral analysis: Axis I, 11.5% of variance; Axis II, 5% of variance. (**B**) Megafloral analysis: Axis I, 3% of variance; Axis II, 2% of variance. Paleocene samples are indicated with blue diamonds, Eocene samples with green triangles, and PETM samples with red squares. PETM samples are compositionally distinct from both Paleocene and Eocene ones and from each other.

The lower flora is dominated by an undescribed, mimosoid legume leaflet and contains leaves similar to "*Artocarpus*" *lessigiana* (Lesquereux) Knowlton, a taxon known from the Paleocene and Eocene of the Denver Basin, Mississippi Embayment, and California

(*29*), locations 650 to 1500 km to the south (Fig. 1 and fig. S2). The upper flora is co-dominated by an undescribed leaf of probable lauralean affinity with a long drip tip, a typical Paleocene lauralean known as "*Ficus*" *planicostata*, and the common late Paleocene–early Eocene platanoid *Macginitiea nobilis* (fig. S3).

Palynofloras extracted from the megafloral sites also have unusual floral composition compared to latest Paleocene and post-PETM samples from the same region (Fig. 3A) (*16*, *17*). Both palynofloras have common, stratigraphically long-ranging, wind-pollinated taxa (such as *Caryapollenites*, *Ulmipollenites*, and *Alnipollenites*), but the lower flora also includes *Brosipollis*, a marker of the early Eocene on the Gulf Coastal Plain (*30*, *31*); *Punctatosporites*, an Eocene index fossil in the Bighorn Basin; and four taxa not previously recorded among ~25,000 grains identified from the late Paleocene and earliest Eocene in the Bighorn Basin (*17*). The upper site contains ?*Lanagiopollis*, cf. *Tricolpites hians*, and *Platycarya swasticoides* (three forms otherwise restricted to the Gulf Coastal Plain); *Triporopollenites granulatus* and *Cycadopites scabratus* (both otherwise found in the Powder River and Williston Basins to the east); the Eocene index *Platycarya platycaryoides*; and four taxa previously unrecorded in the Bighorn Basin including cf. *Bombax* (*30*–*33*). As in the Powder River Basin, *P. platycaryoides*, which migrated to North America from Europe, does not appear until the upper part of the PETM, suggesting it may have not have colonized middle latitudes until climate began to cool late in the event (*19*).

The taxa found in these PETM floras are otherwise unknown from the northern Rocky Mountain region, and the four palynomorphs and one leaf type noted above document northward range extensions from the Gulf Coastal Plain and from Colorado (Fig. 1 and table S4). PETM occurrences of these taxa 650 to 1500 km north of their Paleocene distributions roughly indicate the magnitude of range extension, although incomplete knowledge of Paleocene distributions means these may be overestimates. Paleogene gradients of temperature with latitude have been estimated at 0.4 to 1°C change in mean annual temperature (MAT) per degree of latitude (*34*, *35*). A temperature increase of 4 to 8°C during the PETM (*36*) should have shifted floral ranges 4 to 20 degrees of latitude (450 to 2200 km) to the north, which agrees with the range extensions we infer.

The combination of immigrants from the south, east, and Europe, along with the persistence of natives, is consistent with species-specific, or "individualistic," response to the PETM, as has been widely reported in late and postglacial floras (*37*). The presence of immigrants in the lowest PETM flora suggests that plant range changes were geologically rapid (<10 ky from the base of the CIE). The absence of a distinctive PETM flora in earlier studies probably reflects inadequate sampling (*17*, *19*) or limited change in floral ranges on isolated land masses (*18*). The appearance during the PETM of both intra- and intercontinental floral immigrants mirrors the pattern seen in the fauna, which includes both intracontinental (*Meniscotherium*) and intercontinental (hyaenodontid creodont) migrants (*14*, *21*).

Paleoclimate. Leaf margin analysis (LMA) (*38*, *39*) of the 23 dicot leaf morphospecies from the two localities yielded a MAT estimate of 19.8 − 3.1°C for the PETM [the error is 1 SD, following Wilf (*39*)]. This is 4.9°C higher than the MAT (15.7 ± 2.4°C) estimated from LMA of floras from the 250-ky interval immediately before the PETM in the same region and 1.6°C higher than the MAT (18.2 ± 2.3°C) for the 400-ky interval after the PETM (*40*). Oxygen isotopic composition of biogenic apatite from the Bighorn Basin indicates even higher temperature during the PETM (26°C) (*35*). Modern riparian and wetland vegetation has a higher proportion of toothed species than terra firma forest, commonly resulting in 2.5 to 7°C underestimates of MAT (*41*, *42*). All the fossil floras used to estimate MAT were deposited in fluvial backswamps or channel margins (*28*); paleotemperature estimates are therefore likely to be uniformly low. However, the ~5°C warming estimated from LMA is consistent with isotopic temperature estimates.

We used leaf area analysis (LAA) (*43*) to estimate mean annual precipitation (MAP) at 123 cm (the standard error of the regression is 177/ 86 cm) for the combined PETM flora. A different regression derived from a modern data set with more dry sites (*44*) yielded an MAP estimate of 120 cm. The marked increase in leaf size from the lower to the upper PETM megaflora (figs. S2 and S3) led us to estimate MAP separately for each site. By using the two regressions (*43*, *44*) we estimate a MAP of 80 114/ 56 cm and 41 cm for the lower flora. MAP estimates for the upper flora were 144 206/ 100 cm and 132 cm. Although the MAP estimate for the lower flora was derived from only six morphospecies, the two with the smallest leaves (nanophyll-microphyll) are also the most abundant, indicating that small-leaved species were local dominants. MAP estimates for the late Paleocene in southern Wyoming average 138 cm (*45*), suggesting that rainfall declined by ~40% near the onset of the PETM then recovered to normal values by late in the event. A warm, wet climate late in the PETM is consistent with the exceptionally thick paleosols preserved from the upper part of the event (Fig. 2) (*46*).

Previous studies of the PETM have yielded mixed evidence for precipitation change. A higher abundance of terrestrial palynomorphs and eutrophic dinoflagellates in nearshore marine sediments has been cited as evidence of more runoff and higher precipitation (*18*, *47*), as has the greater magnitude of the CIE in pedogenic carbonate nodules than in marine carbonates (*48*). In contrast, the continental record of the PETM in Spain suggests a persistently or seasonally dry climate (*49*), and a possible PETM section in southern England has exceptional amounts of fossil charcoal (*50*).

Without wider geographic coverage, we do not know if the short period of dry climate we infer at the onset of the PETM is regional or global. However, even if it was confined to the northern Rocky Mountain region, it could have had an important positive feedback on climate by increasing the likelihood of burning in the extensive upper Paleocene peats and coals of the Powder River Basin (*9*).

Conclusion. The PETM provides an important analog to present-day anthropogenic global warming, because the two episodes are inferred to have similar rates and magnitudes of carbon release and climate change (*6*). In this context, it is notable that terrestrial floras underwent rapid (within ~10 ky), individualistic range change during the PETM, including both intra- and intercontinental migration. Plant range changes of similar scale may occur with anthropogenic climate change. Fossils revealing this dramatic, transient, floral response to PETM warming eluded years of focused searching, suggesting that other such short-term shifts in floral composition remain to be uncovered from the "deep time" record of ecological change.

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References and Notes

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Obestatin, a Peptide Encoded by the Ghrelin Gene, Opposes Ghrelin's Effects on Food Intake

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Ghrelin, a circulating appetite-inducing hormone, is derived from a prohormone by posttranslational processing. On the basis of the bioinformatic prediction that another peptide also derived from proghrelin exists, we isolated a hormone from rat stomach and named it obestatin—a contraction of obese, from the Latin "obedere," meaning to devour, and "statin," denoting suppression. Contrary to the appetite-stimulating effects of ghrelin, treatment of rats with obestatin suppressed food intake, inhibited jejunal contraction, and decreased body-weight gain. Obestatin bound to the orphan G protein–coupled receptor GPR39. Thus, two peptide hormones with opposing action in weight regulation are derived from the same ghrelin gene. After differential modification, these hormones activate distinct receptors.

The increasing prevalence of obesity is a global problem. Body weight is regulated in part by peptide hormones produced in the brain or gut or both (*1*). Earlier studies on synthetic and peptidyl growth hormone (GH) secretagogues (*2*–*4*) led to the identification of a specific G protein–coupled receptor (GPCR), the GH secretagogue receptor (GHSR) (*5*, *6*), and subsequently to the discovery of its endogenous ligand, ghrelin (*7*), a gut-derived circulating hormone that stimulates food intake (*4*, *8*).

Human ghrelin, a 28 amino acid peptide, is derived by posttranslational cleavage from a prepropeptide of 117 residues. On the basis of bioinformatic searches of putative hormones derived from the prepropeptides of known peptide hormones, we identified a ghrelin-associated peptide. We searched GenBank for orthologs of the human ghrelin gene and compared proghrelin sequences from 11 mammalian species. In addition to the known ghrelin mature peptide, which immediately follows the signal peptide, we identified another conserved region that was flanked by potential convertase cleavage sites (fig. S1, underlined). This region encodes a putative 23–amino acid peptide, with a flanking conserved glycine residue at the C terminus, suggesting that it might be amidated (*9*). We named this ghrelin-associated peptide obestatin.

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Characterization of endogenous obestatin. To detect endogenous obestatin, we prepared a synthetic obestatin peptide and performed radioimmunoassays on rat-tissue extracts with obestatin-specific antibodies. As shown in Fig. 1A, the stomach extract displaced I^{125}-obestatin binding to the obestatin antibodies. Obestatin-like activities from stomach extracts were purified. Immunoreactive (ir) obestatin was eluted in a Sephadex G-50 gel permeation column (Amersham Biosciences, Piscataway, NJ) with estimated sizes of 2.6 and 1.5 kilodaltons (kD), distinct from the elution position of mature ghrelin (Fig. 1B). We subjected peak 1 (2.6 kD) fractions to ion-exchange fast protein liquid chromatography (FPLC). A single peak of ir obestatin was eluted (Fig. 1C) and shown by mass spectrometry and Edman sequencing to contain a peptide with a molecular mass of 2516.3 (Fig. 1D) and with a sequence of FNAPFDVGIKLSGAQYQQIG-XX (*10*). Combined with molecular-weight determination, the full sequence of the purified peptide was predicted to be FNAPFDVGIKLSGAQYQQHGRAL-NH_2, consistent with the obestatin sequence deduced from rat ghrelin cDNA. In addition, mass spectrometric analyses suggested that peak 2 (1.5 kD) represented the last 13 residues of amidated obestatin, indicating further processing.

To investigate differential secretion of ghrelin and obestatin in vivo, we fasted adult male rats for 48 hours before refeeding. Consistent with earlier findings (*11*), fasting led to a major increase in serum ghrelin levels, whereas subsequent refeeding for 2 hours by allowing animals free access to food or drinking water containing dextrose decreased circulating ghrelin (Fig. 1E). In contrast, serum levels of obestatin determined by a radioimmunoassay were constant in the different treatment groups.

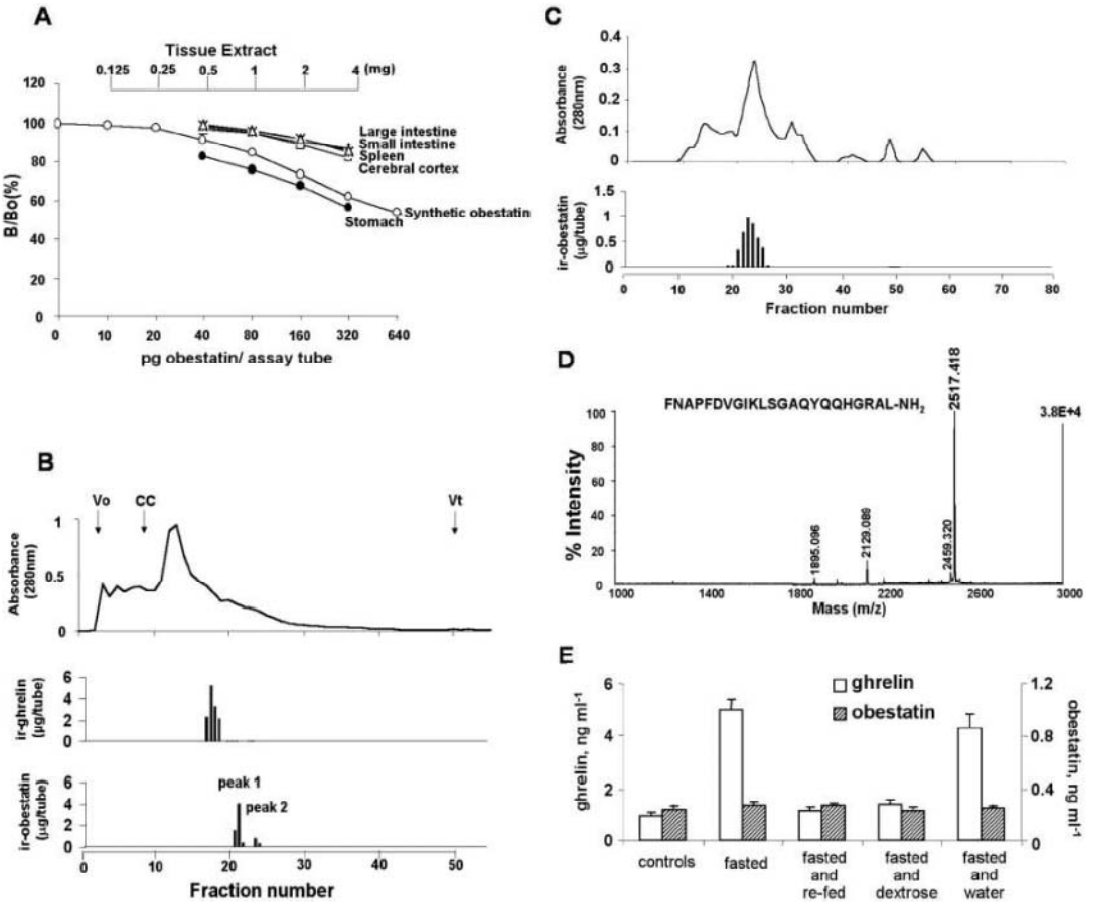

Fig. 1. Characterization of endogenous obestatin. (**A**) Competition of I^{125}-obestatin binding to obestatin antibodies by tissue extracts. I^{125}-obestatin was incubated with obestatin antibodies with or without different dilutions of tissue extracts and the obestatin standard. pg, picograms of; B, bound; Bo, total bound. (**B**) Gel permeation chromatography of obestatin in stomach extracts. Stomach tissues from 30 rats were extracted and eluted from a Sep-Pak C-18 column before they were loaded onto a Sephadex G-50 column. The column was calibrated with blue dextran (v_o), cytochrome c (cc), and potassium chromate (v_t). Peak 1, detected by obestatin antibodies, represents the putative obestatin peptide, and peak 2 represents an obestatin fragment. (**C**) Ion exchange FPLC analysis of peak 1 fractions monitored by the obestatin immunoassay. (**D**) Peptide mapping using mass spectrometry and the predicted amino acid sequence of rat obestatin. *m*, mass; z, charge. (**E**) Serum levels of ghrelin and obestatin during fasting and refeeding. Adult male rats (n = 5 animals per group) were fasted for 2 days. After fasting, some animals were allowed access to food, dextrose solution, or water for 2 hours before the amount of serum hormone was determined using specific radioimmunoassays. Error bars are mean ± SEM.

Obestatin suppression of food intake and gastrointestinal functions. We next synthesized amidated human obestatin and tested its effect on food intake in adult male mice. Intraperitoneal injection of obestatin suppressed food intake in a time- and dose-dependent manner (Fig. 2A). Intracerebroventricular treatment with obestatin also decreased food intake (Fig. 2B), similar to the anorexigenic effect of the synthetic melanocortin agonists MTII (*12*). In contrast, treatment with the nonamidated obestatin (NA-obestatin) was less effective. We also investigated the effect of obestatin, ghrelin, or vehicle alone on body weight in adult male rats. Treatment with ghrelin (1 μmol per kg body weight, three times daily) increased body weight, whereas the same dose of obestatin suppressed body-weight gain (Fig. 2C). Serum leptin levels were not affected after treatment with either obestatin or ghrelin (fig. S2), suggesting minimal modulation of body-fat content. Furthermore, treatment with obestatin led to a sustained suppression of gastric emptying activity (Fig. 2D). In vitro, isometric force measurement demonstrated that obestatin treatment decreased the contractile activity of jejunum muscle strips and antagonized the stimulatory effect of ghrelin (Fig. 2E) (*13*). The observed inhibition of jejunal contraction may trigger an afferent vagus signal to induce a central satiety response. Unlike ghrelin, obestatin did not increase GH secretion by cultured rat pituitary cells (fig. S3).

Obestatin is the cognate ligand for GPR39. Experiments with crude plasma-membrane preparation of rat jejunum revealed that I^{125}-obestatin bound to jejunal preparations with a high affinity (dissociation constant K_d = 4 nM), and this binding was not competed by ghrelin, motilin, neurotensin, or neuromedin U (fig. S4). Furthermore, NA-obestatin and truncated (des1-10)obestatin showed a lower binding affinity than did obestatin. I^{125}-obestatin also bound to the pituitary, stomach, ileum, and hypothalamus, but less so to other tissues (fig. S4).

We hypothesized that obestatin interacts with an orphan GPCR, and we tested obestatin binding to Chinese hamster ovary (CHO) cells transfected with ~30 individual orphan receptor cDNAs. I^{125}-obestatin interacted with high affinity (K_d = 1 nM) to the orphan receptor GPR39, which belongs to the ghrelin receptor subfamily (Fig. 3A) (*14*, *15*). I^{125}-obestatin binding to GPR39 was competed by obestatin but not by ghrelin or several other brain/gut hormones including motilin, neurotensin, or neuromedin U (Fig. 3B). In addition, NA-obestatin and truncated (des1-10)obestatin had a lower affinity for GPR39 than did obestatin. In CHO cells overexpressing GPR39, treatment with obestatin stimulated cyclic adenosine monophosphate (cAMP) production, whereas treatment with ghrelin or motilin was ineffective (Fig. 3C). Consistent with the reported activation of the serum response element (SRE) by constitutive active GPR39 (*14*), hormonal treatment of CHO cells cotransfected with GPR39 and a SRE promoter-luciferase construct led to obestatin but not

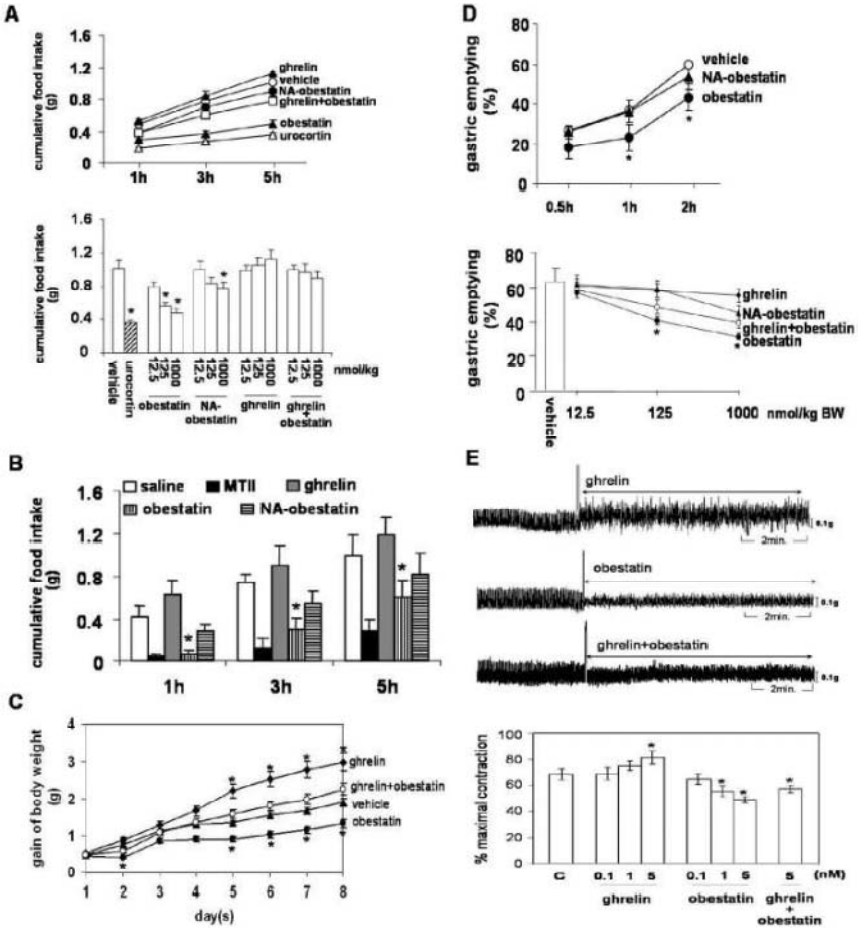

Fig. 2. Regulation of gastrointestinal functions by obestatin. (**A**) Suppression of cumulative food intake after intraperitoneal treatment with obestatin, NA-obestatin, and/or ghrelin. The upper panel shows treatment with different peptides at 1 μmol per kg body weight; the lower panel shows dose response at 5 hours after treatment. Mice injected with urocortin served as positive controls. (**B**) Suppression of cumulative food intake after intracerebroventricular injection of obestatin. Peptides were injected at 8 nmol per kg body weight. Mice injected with MTII served as positive controls. (**C**) Treatment with obestatin suppressed body-weight gain. (**D**) Suppression of gastric emptying activity by obestatin. The upper panel shows treatment with different peptides at 1 μmol per kg body weight; the lower panel shows dose-response relationship at 2 hours after treatment. (**E**) Treatment with obestatin suppressed the contractile activity of jejunum muscle strips and the stimulatory effect of ghrelin. Representative tracing (upper panel) and percentage of maximal responses (lower panel) are shown. Asterisks indicate $P < 0.05$ versus controls (C). Differences between treatment groups were analyzed using analysis of variance and Student's *t*-test.

ghrelin or motilin signaling (Fig. 3D). Similar stimulation of cAMP production and the SRE promoter by obestatin was found when GPR39 was overexpressed in HEK293T cells (fig. S5). Although CHO cells expressing GHSR did not respond to treatment with obestatin or ghrelin, cotransfection with a chimeric Gsq protein, which is capable of switching Gq-mediated signaling to Gs proteins (*16*), led to cAMP increases induced by ghrelin but not obestatin (Fig. 3E). Likewise, cells expressing the Gsq protein and the motilin receptor responded to treatment with motilin but not obestatin (Fig. 3F). Cross-linking studies further demonstrated that I^{125}-obestatin bound to recombinant GPR39, forming a high molecular-weight complex (fig. S6). Real-time reverse-transcription polymerase chain reaction (RT-PCR) analyses indicated that GPR39 is expressed in the jejunum, duodenum, stomach, pituitary, ileum, liver, hypothalamus, and other tissues (Fig. 3G), consistent with obestatin binding studies.

Discussion. Ghrelin is implicated in meal initiation and body-weight regulation. Chronic ghrelin administration increases food intake and decreases energy expenditure, thus causing weight gain. In contrast to ghrelin, which causes hyperphagia and obesity in rats (*17*), obestatin appears to act as an anorexic hormone by decreasing food intake, gastric emptying activities, jejunal motility, and body-weight gain. Mutant mice with a deletion of the ghrelin gene did not show impaired growth or appetite (*6*, *18*), most likely because these animals lacked both orexigenic ghrelin and anorexic obestatin. Indeed, transgenic mice bearing the preproghrelin gene under the control of the chicken β-actin promoter produced high levels of inactive des-acyl ghrelin but exhibited lower body weights (*19*), most likely due to excessive obestatin biosynthesis.

The discovery of amidated obestatin and its cognate receptor underscores the power of comparative genomic analyses in the postgenomic era. A peptide derived from the 66 C-terminal amino acids of proghrelin, named C-ghrelin, was detected in human circulation, and its serum levels were elevated in patients with heart failure (*20*). Although the antibodies used to detect C-ghrelin overlap with obestatin by 13 residues, the exact chemical nature and function of the circulating C-ghrelin remain unclear.

Our finding that two peptide hormones derived from the same proprotein act through distinct receptors and exert opposing physiological actions highlights the importance of posttranslational regulatory mechanisms. Thus, monitoring of ghrelin transcript levels does not accurately reflect the secretion of these two polypeptides. After removal of the signal peptides from prepropeptides, convertases cleave prohormones at mono- or dibasic residues (*21*). In processed peptides with a C-terminal glycine, the residue is further amidated (*9*). Similar to the importance of posttranslational amidation for obestatin bioactivity, ghrelin also requires acylation on its serine-3 residue for bioactivity (*7*).

Ghrelin binds to GHSR, which belongs to the subgroup of type A GPCRs consisting of GPR39 and receptors for ghrelin and motilin (*22*). Our discovery that obestatin is the cognate ligand for GPR39 suggests that GHSR and GPR39 could have evolved from

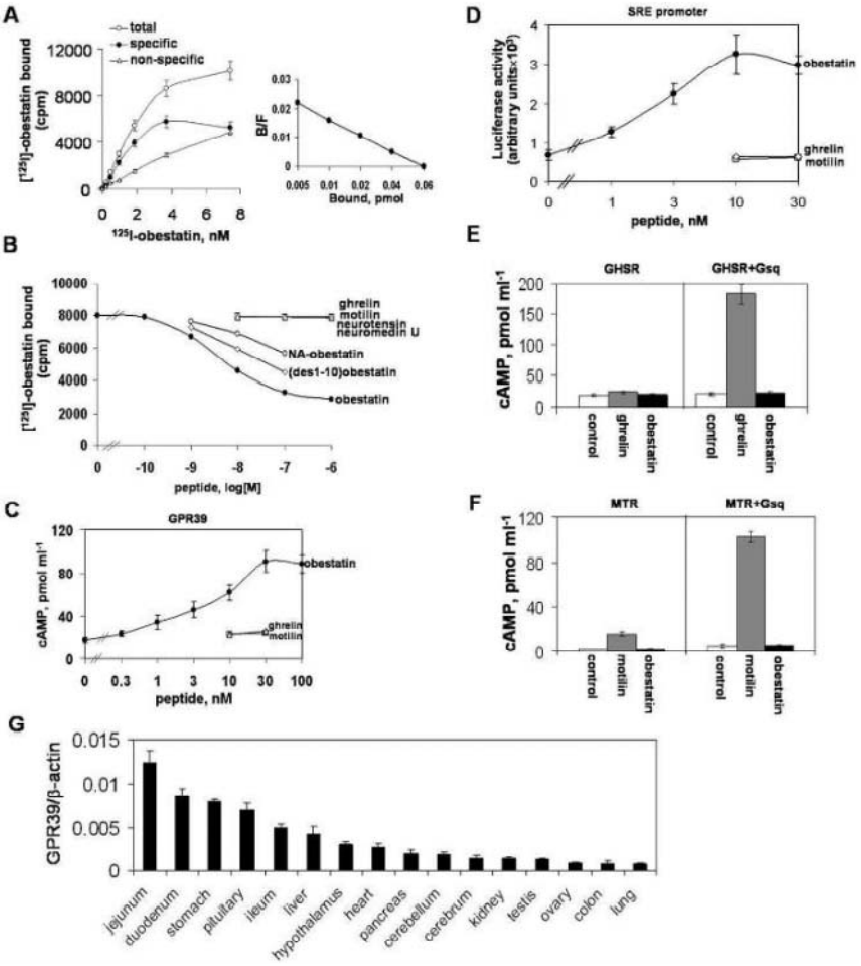

Fig. 3. Obestatin activates the orphan receptor GPR39. **(A)** High-affinity binding of I^{125}-obestatin to CHO cells overexpressing GPR39. Saturation and Scatchard plots are shown. **(B)** Hormonal specificity of I^{125}-obestatin binding to GPR39. Peptides listed were tested separately. **(C)** Obestatin, but not ghrelin or motilin, stimulated cAMP production. **(D)** Obestatin activation of the SRE-luciferase reporter. **(E)** Ghrelin, but not obestatin, stimulated cAMP production in cells transfected with GHSR and the chimeric Gsq protein. **(F)** Motilin, but not obestatin, stimulated cAMP production in cells transfected with the motilin receptor (MTR) and the chimeric Gsq protein. **(G)** Real-time RT-PCR analyses of GPR39 transcript levels in diverse tissues. Data are the mean ± SEM of triplicates.

a common ancestor but diverged in their functions, thus maintaining a delicate balance of body-weight regulation. This scenario is similar to the divergent and sometimes opposing actions of two paralogous corticotropin-releasing hormone receptors and their ligands in the regulation of adaptive stress responses (*23*–*25*).

In addition to roles in meal initiation, weight regulation, and gastrointestinal activity, ghrelin also regulates the pituitary hormone axis, carbohydrate metabolism, and various functions of the heart, kidney, pancreas, adipose tissues, and gonads (*26*). Because ghrelin mRNA was found in almost all human tissues analyzed (*27*), the identification of obestatin derived from the same gene product as ghrelin provides a basis for future elucidation of the differential posttranslational processing and modification of these two peptides. A better understanding of the roles of ghrelin and obestatin in the intricate balance of energy homeostasis and body-weight control may be essential for the successful treatment of obesity.

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A Thiolate-Ligated Nonheme Oxoiron(IV) Complex Relevant to Cytochrome P450

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Thiolate-ligated oxoiron(IV) centers are postulated to be the key oxidants in the catalytic cycles of oxygen-activating cytochrome P450 and related enzymes. Despite considerable synthetic efforts, chemists have not succeeded in preparing an appropriate model complex. Here we report the synthesis and spectroscopic characterization of $[Fe^{IV}(O)(TMCS)]^{+}$ where TMCS is a pentadentate ligand that provides a square pyramidal $N_4(SR)_{apical}$ ligand environment about the iron center, which is similar to that of cytochrome P450. The rigidity of the ligand framework stabilizes the thiolate in an oxidizing environment. Reactivity studies suggest that thiolate coordination favors hydrogen-atom abstraction chemistry over oxygen-atom transfer pathways in the presence of reducing substrates.

Thiolate-ligated oxoiron(IV) centers are thought to be the key oxidants in the catalytic cycles of oxygen-activating iron enzymes, such as cytochrome P450 (P450) (*1*, *2*), NO synthase (NOS) (*3*), and isopenicillin N synthase (*4*). Because of the physiological importance of the heme-containing P450 and NOS, many biophysical and computational studies have sought to elucidate the nature of the oxidizing species compounds I and II, which correspond, respectively, to intermediates with formal iron-oxidation states two and one above the resting iron(III) state (*2*, *3*, *5*–*7*). However, these studies have not yet provided conclusive evidence for the presence of thiolate-ligated oxoiron(IV) species in the reaction cycles. For over 30 years, small-molecule complexes synthesized as active-site models have advanced our understanding of the P450 cycle (*8*, *9*); despite these efforts, the synthesis of an oxoiron(IV) porphyrin complex with a thiolate ligand has not yet been achieved.

A central question in these systems is how cysteinate coordination might influence the reactivity of the oxoiron(IV) unit. Because of the absence of a confining pocket of the protein, it is very difficult to lock a thiolate ligand into a geometry that affords adequate stability in an oxidizing environment, because the sulfur center is generally at least as susceptible as the iron center to oxidative attack. Recently, $[Fe^{II}(TMCS)](PF_6)$ (Scheme 1, complex **1**) (where TMCS is a monoanion of 1-mercaptoethyl-4,8,11-trimethyl-1,4,8,11-tetraaza cyclotetradecane), a nonheme iron complex with a square pyramidal $N_4(SR)_{apical}$ ligand set, was synthesized in one of our laboratories as a model for the iron(II) active site of superoxide reductase (*10*). We reasoned that this framework may be sufficiently rigid to support the coordination of the pendant thiolate to the iron center trans to an oxo group. The synthesis of such a complex (**2**) is reported here. Moreover, an analogous complex, **3**, in which the pendant mercaptoethyl group is replaced by methyl, forms a stable oxoiron(IV) complex that has been characterized crystallographically (*11*). Complex **3** functions as an ideal control from which to infer the precise impact of thiolate coordination on the structure and reactivity of the oxoiron(IV) unit in **2**.

The reaction of **1** with 3 to 5 equivalents of H_2O_2 at −60°C in methanol elicits the formation of a deep blue complex **2**, with

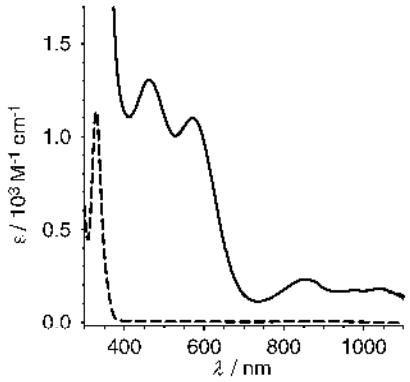


Fig. 1. Electronic spectral changes in the conversion of 1.04 mM **1** (dashed line) to **2** (solid line) in methanol at −40°C by the addition of one equivalent *m*CPBA in the presence of 6 equivalents of potassium *tert*-butoxide.

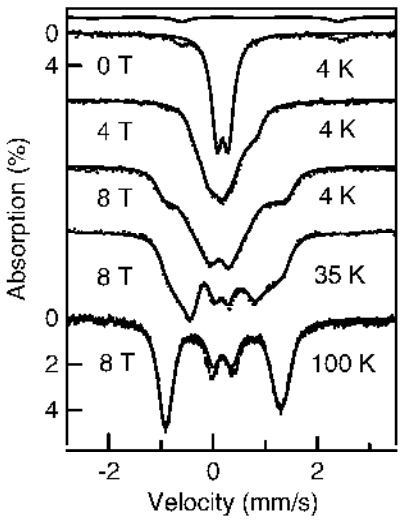
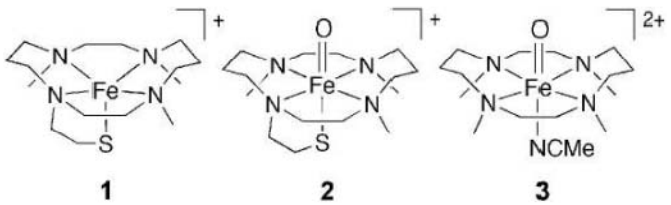


Fig. 2. Mössbauer spectra of a frozen methanol solution of **2** recorded at temperatures and applied fields indicated. Spectral simulations are based on Eq. 1 using the parameters listed in Table 1. Spectra were simulated in the slow (at 4.2 K) and fast (for T > 30 K) spin fluctuation limit. The applied field was directed parallel to the observed γ-radiation. The doublet drawn above the topmost experimental spectrum (0 T, 4 K) represents a 7% contribution from a residual amount of **1** in the sample of **2**.



Scheme 1.

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intense visible absorption features at 460 and 570 nm and weaker near-infrared (IR) bands at 850 and 1050 nm (Fig. 1). Although this species is quite stable at −60°C, it decays at higher temperature with all four bands decreasing at the same rate. The features in the near-IR region resemble those observed for recently identified nonheme oxoiron(IV) complexes (*11*–*13*), including **3**, which correspond to d-d transitions of an $S = 1$ metal center (where S is the spin quantum number) (*14*). Initial Mössbauer studies confirmed the formation of an iron(IV) complex (see below), but with only 40% yield. However, in subsequent experiments, **2** could be generated with greater than 90% yield by using one equivalent of *m*-chloroperbenzoic acid (*m*CPBA) as oxidant in the presence of excess base. High-resolution electrospray mass spectral studies of **2** revealed only one prominent ion at a mass/charge (m/z) ratio of 373.1724, with a mass value that corresponds exactly with its formulation as $[Fe(O)(TMCS)]^+$ (fig. S1).

Figure 2 shows Mössbauer spectra of ^{57}Fe-enriched complex **2** recorded at 4.2, 35, and 100 K in applied magnetic fields as indicated. In zero field, **2** exhibits a doublet (accounting for ~93% of the total Fe) with quadrupole splitting $\Delta E_Q = 0.22$ mm/s and isomer shift $\delta = 0.19$ mm/s (relative to Fe metal at 298 K). The remainder of the iron gives rise to a doublet that is attributable to starting material **1** ($\Delta E_Q = 3.0$ mm/s and $\delta = 0.90$ mm/s). The applied field spectra show that **2** is an integer spin paramagnet, with parameters similar to those of **3** (*11*). The δ value of **2** is only slightly larger than that of **3** ($\delta = 0.17$ mm/s). Thus, although the δ value supports the assignment of **2** as an iron(IV) complex, it cannot be used to establish thiolate coordination to the $S = 1$ Fe^{IV}=O state. [In contrast, thiolate ligation strongly affects δ in the high-spin iron(II) state (*15*).] However, the effect of thiolate binding to the oxoiron(IV) center is manifested in the much smaller ΔE_Q of **2** relative to that of **3**.

Table 1. Experimental (exp) and calculated (calcd) parameters for **2** and **3**. Numbers in parentheses indicate the error in the last significant digit. nd, no data; η, asymmetry parameter of the electric field gradient (EFG) tensor; r, bond distance.

Complex	D (cm^{-1})	E/D	$(A_x, A_y, A_z)/g_N\beta_N$ (T)	ΔE_Q (mm/s)	η	δ (mm/s)	r_{Fe-O} (Å)	r_{Fe-N} (Å)	r_{Fe-S} (Å)
2 (exp)	35(3)	0	−23(2),−22(2),−5(2)	−0.22(2)†	≈0†	0.19(1)	1.70‡	2.09‡	2.33‡
2 (calcd)	36	nd	−23.3*, −22.2*, −4.8*	−0.37†	0.6†	0.20	1.68	2.13	2.39
3 (exp)	28	0	−25, −20, −3	1.23	0.5	0.17	1.646§	2.09§	–
3 (calcd)‖	27	nd	−20.5, −20.1, −4.3	1.25	0.1	0.175	1.64	2.10	–

*Sum of the calculated spin-dipolar contribution (traceless) and the experimental value for the isotropic contribution, $A_{iso}/g_N\beta_N = -16.8$ T; $A_{iso} = (A_x + A_y + A_z)/3$. †The ZFS and A tensor are essentially axial, with z within 8 degrees along the Fe–O axis according to DFT calculations (22). The experimental EFG tensor is axial, with the major component anywhere in the xy plane, which is roughly the plane defined by the four N ligands; $\Delta E_Q < 0$. We have quoted ΔE_Q and η in the conventional way, i.e., in a coordinate system for which $0 \le \eta \le 1$. The major component of the calculated EFG is also in the xy plane. ‡This work, from EXAFS analysis (table S1). §See (*11*). ‖Schöneboom *et al.* (*26*) have recently reported similar values for **3**.

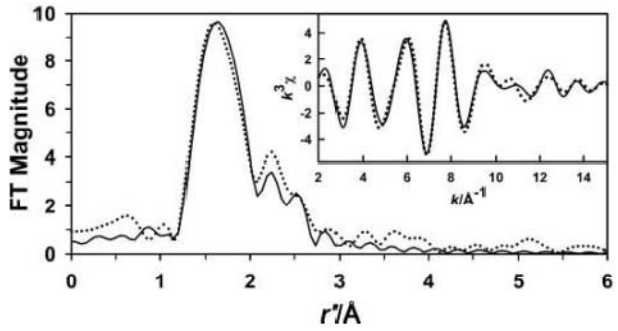

Fig. 3. r'-space and k-space (inset) EXAFS data for **2** obtained in frozen methanol solution. Experimental data (dotted line) and fits to the data (solid line) are shown. Fourier transform (FT) range (k): 2 to 15.07 Å^{-1} (0.12 Å resolution). Back-transformation range (r'): 0.77 to 3.18 Å. See table S1 for a summary of the fitting protocol. Best fit shown consists of 1 O at 1.70(2) Å, 3 N/O at 2.09(2) Å, 1 S at 2.33(2) Å, and 4 C at 2.95(2) Å.

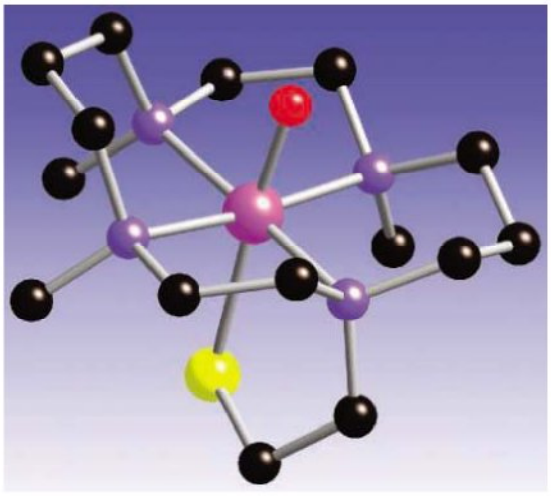

Fig. 4. Geometry optimized structure of **2** based on DFT calculations (22). Black, carbon; red, oxygen; yellow, sulfur; purple, iron; and blue, nitrogen.

The spectra of Fig. 2, together with data obtained at different temperatures and applied fields, were simulated with the $S = 1$ spin Hamiltonian

$$H = D(S_z^2 - 2/3) + E(S_x^2 - S_y^2) + 2\beta\mathbf{B}\cdot\mathbf{S} \quad \mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I} - g_N\beta_N\mathbf{B}\cdot\mathbf{I} + H_Q \quad (1)$$

where H_Q describes the quadrupole interactions, D and E represent the zero-field splitting (ZFS) parameters, **A** is the magnetic hyperfine tensor, **I** is the nuclear spin operator, **B** is the magnetic field, β is the Bohr magneton, and $g_N\beta_N$ is the nuclear gyromagnetic ratio. For the simulations, we kept all tensors in the same principal axis frame. These values are summarized in Table 1 and are compared with those reported previously for **3**.

X-ray absorption spectroscopy experiments offered further insight into the structure of **2**. This complex gives rise to a sharp pre-edge feature with an area of 18(2) arbitrary units (AU), where the number in parentheses indicates the uncertainty in the last significant digit (*16*). This feature arises from 1s-to-3d transitions, the intensities of which reflect deviations of the metal center from centrosymmetry (*17*). Other $S = 1$ oxoiron(IV) complexes have been found to show similarly intense pre-edge features, with areas ranging from 24 to 32 AU (*18*). The smaller value associated with **2** relative to that of **3** (30 AU) presumably reflects differences in bonding that may be attributed to the stronger σ and π donating properties of the thiolate group in **2** relative to the more π-acidic $NCCH_3$ ligand in **3**.

The extended x-ray absorption fine structure (EXAFS) data for **2** can be fit well with the following scatterers: 1 O at 1.70(2) Å, 3 N/O at 2.09(2) Å, 1 S at 2.33(2) Å, and 4 C at 2.95(2) Å (Fig. 3 and table S1), revealing an iron center with both an oxo and a thiolate ligand.

Coordination of the axial thiolate lengthens the Fe–O bond slightly in **2** relative to that found in the crystallographically characterized **3**, but it does not affect the average Fe N distance (Table 1). The Fe–S distance found in **2** is intermediate between that of its precursor complex **1** [2.297(3) Å] (*10*) and that determined by EXAFS for chloroperoxidase compound II (2.37 Å) (*19*), but is significantly shorter than the Fe S bonds computed for cytochrome P450 compounds I (2.6 Å) and II (2.5 Å) (*20*, *21*). The shorter Fe S bond observed for **2** may reflect the higher effective charge of the iron center because of its uncharged equatorial N_4 ligand set. Complex **2** has Fe=O and Fe–S bond lengths that closely match those of a putative P450 intermediate produced by cryophotoreduction of oxyP450 crystals, as deduced from its 1.9 Å resolution crystal structure (*5*).

To gain further insight into the electronic structure of **2**, we performed density functional theory (DFT) calculations (22). In the lowest-energy calculated structure (Fig. 4 and Table 1), the lengthening of the Fe–O bond to 1.68 Å, relative to that in **3**, is in excellent agreement with the EXAFS analysis. The π-basic thiolate ligand donates electron density to the iron(IV) center and competes with the oxo group for the metal d_π orbitals, thereby weakening the Fe–O bond. In contrast, the π-acidic MeCN ligand in **3** has a backbonding interaction with the iron(IV) center that strengthens the Fe=O bond. The very large zero-field splitting (ZFS) of **2** results predominantly from spin-orbit mixing between the $S = 1$ ground state and a very low lying $S = 2$ manifold (22) at lower energy (≈2000 cm^{-1}) than in **3** (≈ 3000 cm^{-1}), facilitating a stronger interaction.

The introduction of the thiolate ligand not only affects the electronic properties of the oxoiron(IV) unit but also has a dramatic effect on its reactivity. At –40°C, both **2** and **3** have extended lifetimes of days for **2** in MeOH and weeks for **3** in MeCN (*11*). Complex **3** reacts readily with PPh_3 by oxo-atom transfer to form $OPPh_3$ and regenerate its iron(II) precursor (*11*), but it is inert toward dihydroanthracene, a hydrocarbon that typically undergoes facile hydrogen-atom abstraction. In contrast, **2** does not react at all with PPh_3 but reacts with even one equivalent of dihydroanthracene in under an hour. For the latter reaction, **2** undergoes a one-electron reduction to a red species (maximum wavelength λ_{max} = 514 nm; molar absorptivity ε = 1400 $M^{-1}cm^{-1}$) (fig. S2) that exhibits a prominent electrospray ionization mass spectroscopy (ESI-MS) ion at m/z = 388.1944, corresponding to $[Fe(TMCS)(OMe)]^+$. We postulate that **2** decays by abstracting a hydrogen atom from dihydroanthracene to form $[Fe^{III}(TMCS)(OH)]^+$, which readily converts to $[Fe^{III}(TMCS)(OMe)]^+$ in MeOH.

Thus, the introduction of the axial thiolate converts the Fe^{IV}–O unit from being an oxo-atom transfer agent (two-electron oxidant) into a hydrogen-atom abstraction agent (one-electron oxidant). This switching effect of the axial thiolate on the reactivity of the oxoiron(IV) unit is much more dramatic than was previously reported for other axial ligand substitutions on the $[Fe^{IV}(O)(TMC)]$ framework (*23*, *24*), as well as for those associated with oxoiron(IV) porphyrin cation radical complexes (*25*). Understanding this switch will require further experimental and computational work. However the unusual effect of the thiolate ligand may provide a compelling rationale for nature's use of the $O = Fe^{IV}$–SR motif in key metabolic transformations that involve the activation of strong C-H bonds.

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Tables S1 to S4
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Direct Visualization of the Formation of Single-Molecule Conjugated Copolymers

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Electrochemical polymerization of two different kinds of thiophene monomers on an iodine-covered gold surface created highly assembled conjugated copolymers with different electronic structures. A scanning tunneling microscope revealed images of several linkage types: diblock, triblock, and multiblock. The single strand of conjugated copolymers exhibited an anomalous swinging motion on the surface. This technique presents the possibility of understanding the copolymerization process from the different monomers on the single-molecular scale and of building single-molecule superlattices on a surface through controlled electropolymerization.

Conjugated copolymers (*1*, *2*), which combine different kinds of molecules with π-electron networks, have useful conductivity properties that can be exploited in devices such as field effect transistors (*3*). However, the limited solubility of these materials has obscured details of the polymerization process. For example, does polymerization proceed in blocks or is it random? Visualization of conjugated copolymers on the single-molecular scale can address these questions.

Manipulated reactions (induced by a scanning probe microscope tip) on surfaces have been demonstrated, such as one-dimensional chain polymerization of diacetylene into polydiacetylene (*4*), the coupling reaction of iodobenzene into the biphenyl (*5*), K atom doping into C_{60} molecules (*6*), and the connection of two different dendronized polymers by ultraviolet light irradiation (*7*). Applications of the electrochemical technique to solutions containing different kinds of monomers or mixed solutions provide a new approach for the production of single-molecule heterowires on surfaces.

Heterojunctions of synthetic conjugated copolymers (*8*) and synthetic conjugated oligomers (*9*, *10*) have been imaged, but clear visualization of the connection of different single-molecule wires is difficult unless the polymers are highly ordered on the surfaces. Here, we used electrochemical epitaxial polymerization (ECEP) to synthesize conjugated copolymers on surfaces so that polymerization could be imaged on the single-molecular scale.

Conjugated polymers can be assembled on a metal surface by means of ECEP with control of the molecule's length, density, and propagation direction (*11*). In this technique, voltage pulses are applied to monomers in an electrolyte solution on an iodine-covered gold substrate [I-Au(111)] (*12*). Two steps are crucial: (i) nucleus formation, in which the oligomers produced in solution adsorb on the I-Au(111), and (ii) stepwise polymerization such that the monomer's cation radicals react with the nucleus to form wires that propagate along the surface's iodine-atom lattice. The iodine-covered surface acts as an adhesive that binds the polythiophene wires. Two kinds of polythiophenes were used as components for creating heterowires and were characterized independently with scanning tunneling microscopy (STM) (*12*). All STM images were taken at a tip bias of −0.2 V with a constant current of 5 pA. Two kinds of thiophene monomers were used as building blocks to create heterowires (Fig. 1, B and E): 3-octyloxy-4-methylthiophene (C8OMT) (*13*) and 3-octyl-4-methylthiophene (C8MT) (*14*). Cyclic voltammogram (*12*) shows that the oxygen atom that is directly bonded to the thiophene moiety in C8OMT affords a lower oxidation potential by 0.4 V relative to C8MT (fig. S1).

Application of the ECEP technique at a given condition to the C8OMT monomer (Fig. 1A) produced linear polythiophene arrays on the I-Au(111) with a maximum length of 100 nm and a height of 3.0 to 3.5 Å, as measured with STM (Fig. 1, B to D). The C8MT-polymer wires were also produced at different ECEP conditions with a maximum length of 50 nm and a height of 1.5 to 2.0 Å (Fig. 1, E to G). In marked contrast to the C8OMT-polymer wires, the C8MT-polymer wires appeared in STM images as a connection of tiny dots, which were spaced at 11.5 Å (Fig. 1, E to G). We observed that isolated single polymers of C8OMT and C8MT in the low density region and those with lengths shorter than 10 nm (*11*) moved easily on I-Au(111) surface. The dots observed at C8MT polymers were not disconnected, even though the polymers moved on the surface. These results strongly suggest that the dot structure is due not to the self-assembled C8MT monomers or its oligomers but to the single-polymer strand. Because of the asymmetric reactivity of the monomer, 3-alkoxy-4-methylthiophenes such as C8OMT are reported to provide the regioregular head-to-tail polythiophenes (*13*, *15*). The same reason might be applicable to the C8MT because of the asymmetrical chemical structure.

These results suggest that the electronic structures of the two kinds of polythiophene differ greatly. The optical absorption spectrum of the chemically synthesized polymers of C8MT in solution showed a highest occupied molecular orbital (HOMO)–lowest unoccupied molecular orbital (LUMO) gap of 3.76 eV, whereas that of C8OMT showed a gap of 2.94 eV (fig. S2). These data are in a good agreement with the reported spectrum of each polymer (*13*, *14*, *16*). Such a large HOMO-LUMO gap of C8MT polymer has been ascribed to thiophene-ring torsion (*17*–*19*) in the main polymer chain, resulting in "short conjugation" of π electrons.

Thiophene ring torsion is the most probable reason that features in the STM image of C8MT-polymer wires appear in the shape of connected dots with nodes (Fig. 1, E and F). The 11.5 Å spacing of dots (Fig. 1G) observed from the STM image of C8MT-polymer wires agrees well with the threefold 3.8 Å spacing (*11*, *20*–*22*) of interthiophene units in polythiophenes (Fig. 1, F and G). Two-dimensional Fast Fourier Transform (FFT) of Fig. 1F represents the line patterns perpendicular to the direction of polymer chain, which provide an accurate periodicity of dots (inset of Fig. 1H). The FFT cross-sectional peak at 0.85 nm^{-1} shows the periodicity to be 11.73 Å (Fig. 1H). This value suggests that torsion might have a periodicity of three thiophene units (Fig. 1I). It

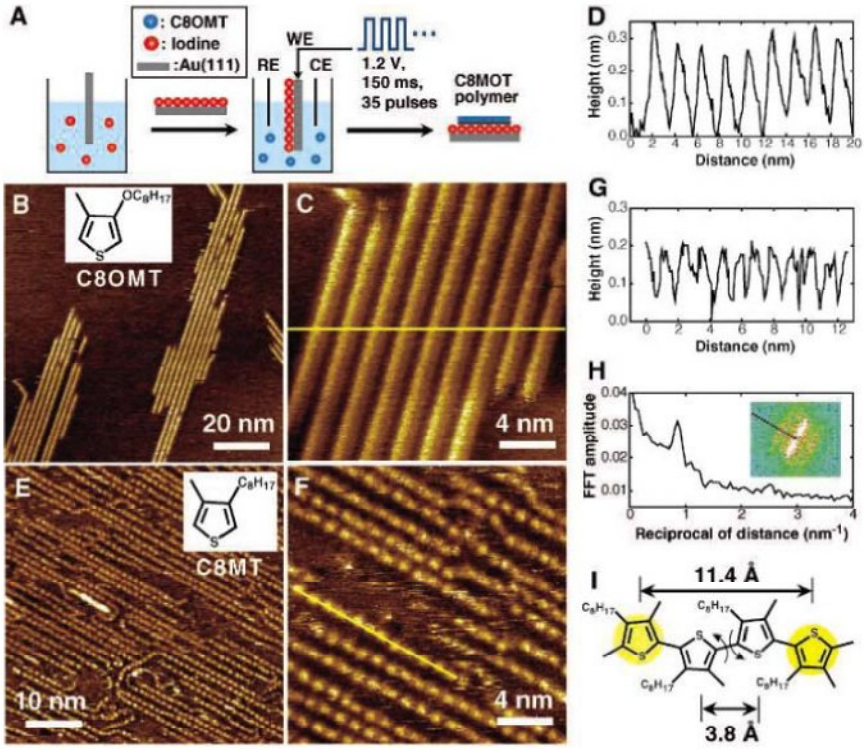

Fig. 1. (**A**) Experimental set-up of ECEP to produce the monopolymer wires, showing working (WE), reference (RE), and counter electrodes (CE). (**B** and **C**) STM images of C8OMT-polymer wires made by applying 35 pulses (1.2 V, 150 ms) to the I-Au(111) substrate in the C8OMT (10 mM)–NBu_4PF_6 (0.1 M) dichloromethane solution. The inset depicts the C8OMT chemical structure. (**D**) Cross section of line shown in (C). (**E** and **F**) STM images of C8MT-polymer wires made by applying 150 pulses (1.4 V, 150 ms) to the I-Au(111) substrate in the C8MT (10 mM)–NBu_4PF_6 (0.1 M) solution. (**G**) Cross section of the line shown in (F). (**H**) Two-dimensional FFT image of (F) (inset) and its cross section. (**I**) Proposed structure of C8MT polymer on the surface. Yellow circles correspond to center of dots shown in the STM images.

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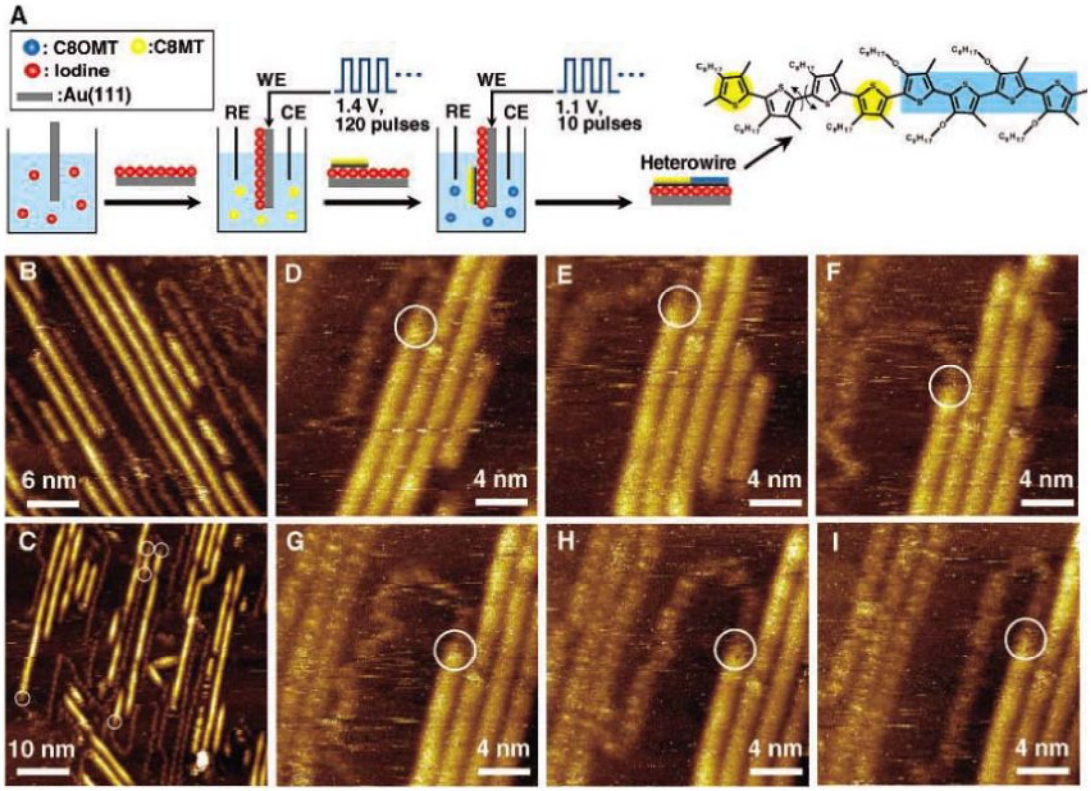

Fig. 2. (A) Experimental setup of multistep ECEP to produce heterowires. (B and C) STM image of I-Au(111) after application of multistep ECEP. Multistep ECEP consists of the first voltage-pulse application (1.4 V, 150 ms, 120 pulses) to a C8MT (10 mM)–NBu_4PF_6 (0.1 M) solution and the second application (1.1 V, 150 ms, 10 pulses) to a C8OMT (10 mM)–NBu_4PF_6 (0.1 M) solution. (D to I) Temporal STM images of I-Au(111) after application of multistep ECEP with the same conditions as (B) and (C), acquired every 2 min. Circles show heterojunctions.

has been reported that the STM height is affected by a barrier height and a transconductance of molecules (*23*, *24*). Observed STM images reflect the electronic structure of the polymer coupled with the geometry. The bright dots in the chains that were visible in STM images probably correspond to the electronic orbitals on planar thiophene rings with high conjugation of π electrons, whereas the nodes (dark regions) should appear as those on distorted thiophene-rings with low conjugation (Fig. 1I). Although the alkyl chains of polymers cannot be imaged because of the high barrier height of C-C bonds, the conjugated chain-to-chain distances of 1.66 nm on average (Fig. 1F) suggest that the alkyl chains of C8MT polymer might interdigitate with the adjacent polymer's alkyl chains. Thus, the torsion model can explain the structures shaped as connected dots in STM images of C8MT-polymer wires.

It has been reported that the distortion of the thiophene ring in quarterthiophene is affected by the medium (crystal or solvent) as well as the intrinsic properties of molecules (*25*). Therefore, we studied the substrate effect to explain why the periodicity of the dot-shaped structure is highly regular. The structure of C8MT polymers fabricated on Au(111) was compared with that on I-Au(111). An STM image of C8MT polymers on Au(111) showed randomly oriented wires with no apparent periodic dot-shaped structure (fig. S3A). The C8OMT polymers on Au(111), as a reference, represent a rodlike structure similar to that on I-Au(111) (fig. S3B). Thus, these results suggest that the highly regular periodic structure of C8MT polymers might be influenced on the surface. The interaction between the C8MT polymer and the iodine atoms plays a crucial role in forming the periodic structure.

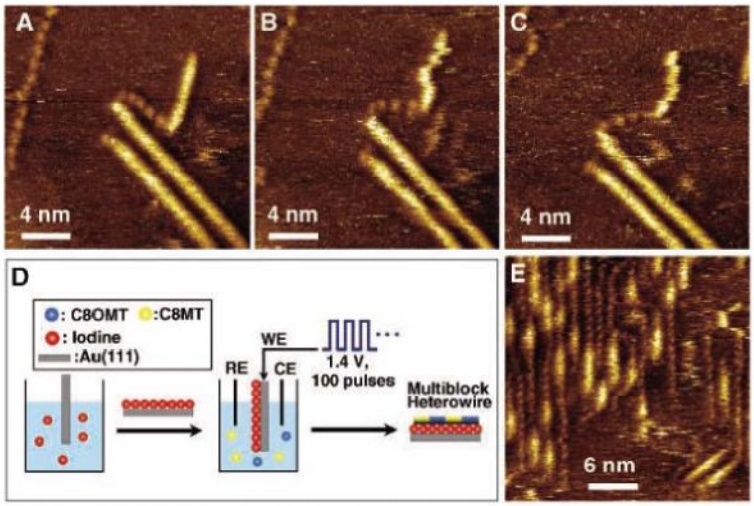

Fig. 3. (A to C) Dynamic STM images of I-Au(111) after application of multistep ECEP, acquired every 2 min. Condition of multistep ECEP are identical to those in Fig. 2. (D) Experimental setup of mixed-solution ECEP method to produce multiblock heterowires. (E) STM image of I-Au(111) after application of mixed-solution ECEP. Mixed-solution ECEP consists of the voltage-pulse application (1.4 V, 150 ms, 100 pulses) to a C8MT (10 mM)–C8OMT (1 mM)–NBu_4PF_6 (0.1 M) solution.

We propose a multistep ECEP technique (*12*) to create single-molecule heterowires (Fig. 2A). This technique comprises two electropolymerization processes in a different monomer solution. In the first process, the voltage pulses to oxidize the monomer were applied to the I-Au(111) in the electrolyte solution containing the C8MT mono-

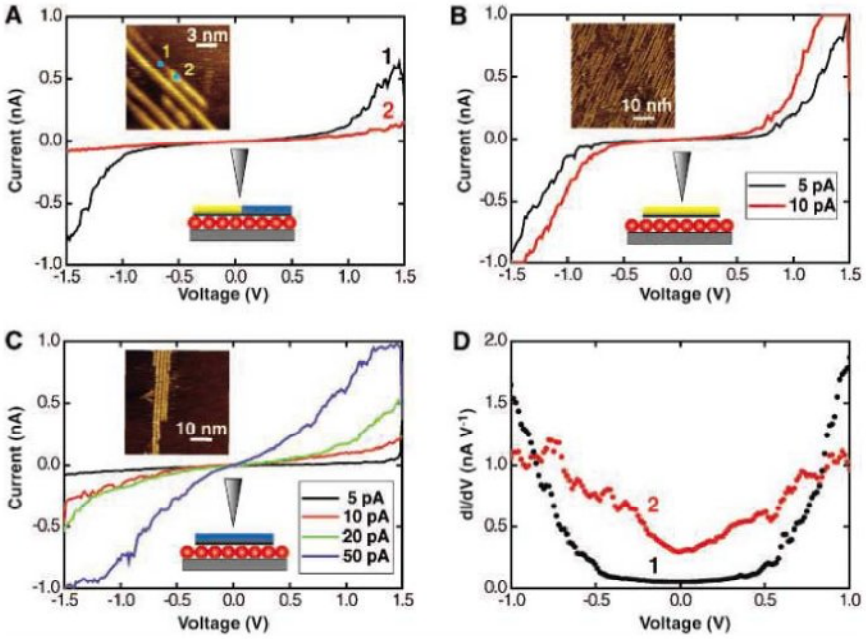

Fig. 4. (**A**) STS curves located on (1) the C8MT-wire part of a heterowire and (2) the C8OMT-wire part. Initial tunneling current was 10 pA. Insets show the STM image with blue dots where the STS measurements were performed and the experimental illustration. (**B**) STS curves of C8MT-polymer wires as a function of initial tunneling current of 5 and 10 pA. (**C**) STS curves of C8OMT-polymer wires as a function of initial tunneling current of 5, 10, 20, and 50 pA. Insets show the STM image of sample and the experimental illustration. (**D**) (1) Differential conductance (*dI*/*dV*) of C8MT-polymer wires (B) from the initial tunneling current of 10 pA and (2) those for C8OMT-polymer wires (C) from the initial tunneling current of 50 pA.

mer. This process produces the C8MT-polymer wires on the substrate. The substrate is then transferred to an electrolyte solution containing C8OMT, and a second process of voltage application oxidizes both the C8OMT in solution and the C8MT polymer on the substrate. The second process might create heterowires in which the C8MT polymers on the substrate link with propagating C8OMT polymers. The STM images for the sample obtained by multistep ECEP depict two independently grown wires in some regions (Fig. 2B). From differences in their shape and height, the observed wires are easily classifiable into two types of polymer blocks. However, other regions showed heterowires with a C8OMT polymer and a C8MT polymer joined together at the ends of respective chains (Fig. 2C).

Sequential STM images show that the heterojunction is created by chemical bonding between the C8OMT-polymer and C8MT-polymer wires. Dynamic STM images taken every 2 min (Fig. 2, D to I) revealed that the C8MT-polymer part swings from the point at heterojunction (circles shown in Fig. 2, D to I), whereas the C8OMT-polymer part is tightly fixed on the surface. If the two wires are not covalently bonded but merely contacted, the C8MT-polymer wire would separate from the C8OMT polymer at the heterojunction. However, the two wires never separated at the heterojunction. These results indicate the evidence of a covalent bond at the heterojunction. Increasing the imaging tunneling current from 5 to 10 pA did not affect the swinging motion of heterowires. Therefore, the swinging motion might not be caused by the tip manipulation but by the thermal-activated polymer diffusion on the surface.

There might be two reasons for the polymer diffusion on the surface. One reason is the binding force of the different polymers with the surface: The C8OMT-polymer wires bind to the I-Au(111) more strongly than do the C8MT wires. Periodic torsion of thiophene rings of C8MT polymer might reduce the interaction with I-Au(111), whereas the coplanar thiophene rings of C8OMT polymers tightly interact with I-Au(111). The second reason is a surrounding effect: the interchain interaction with neighbor polymers. The C8MT-polymer part of heterowire (Fig. 2, D to I) is almost isolated from the C8OMT-polymer array because of its bending chain. This situation makes the C8MT-polymer part move easily on the surface because it has no interaction with the C8OMT-polymer array. Once the C8MT-polymer chains on surface have aligned parallel to the adjacent chains of C8OMT or C8MT polymers, these had a tendency to stabilize (some wires in Fig. 2, B and C). Interaction between the alkyl chain of the polymer and the interdigitated one plays an important role in the stability of the polymer on the surface (*11*). Different binding forces of two polymers might originate from an interaction between the polymer wires and the I-Au(111).

In some cases, we observed not only diblock wires but also triblock wires (Fig. 3, A to C). These STM images show a triblock structure in which a C8MT-polymer wire is sandwiched between two C8OMT wires. These images also represent the motion of the C8MT-polymer part linked with the short C8OMT polymer, for which the long C8OMT-polymer part adsorbs strongly on the surface. The short length of the isolated wire might be the reason for the motion of the C8MT-polymer part linked with the short C8OMT polymer. When ECEP was used with a mixed solution containing C8OMT and C8MT (Fig. 3D), multiblock heterowires were still produced rather than random copolymers (Fig. 3E). Although the multiblock structures were sometimes visible by a multistep ECEP (Fig. 2C shows some junctions), the mixed-solution method produced these structures efficiently, because such structures frequently appeared in many different locations. These results indicate that the polymerization process of conjugated copolymer in the mixed solution is not random but block polymerization, and that monomers react preferentially with each other rather than with different monomers.

The electronic structure of heterowires was investigated with scanning tunneling spectroscopy (STS) (*12*). The current-voltage (IV) curves of heterowires from an initial tunneling current of 10 pA are depicted in Fig. 4A. The C8MT-polymer parts of heterowires show a larger HOMO-LUMO gap than those at C8OMT-polymer parts. Observed STS data show almost symmetrical IV curves in the present bias range. Reported STS of oligothiophene also shows symmetrical IV curves (*26*). Typical IV curves of C8MT and C8OMT polymers (Fig. 4, B and C, respectively) show that changing the initial tunneling current regulates the tip-sample distance. The differential conductance (*dI*/*dV*) of Fig. 4B (C8MT polymer) from the initial tunneling current of 10 pA and that of Fig. 4C (C8OMT polymer) from 50 pA are plotted in Fig. 4D. Although the current in the IV curves sometimes fluctuated because of the measurement conditions, the shapes of curve were reproducible. The IV curves of C8MT polymers (Fig. 4B) and C8OMT polymers (Fig. 4C) were the same as those for the C8MT-polymer blocks and the C8OMT-polymer blocks of the heterowires, respectively. Thus, the electronic properties of the heterowires of each polymer are the same as those of the wires of each homopolymer. The HOMO-LUMO gap of the C8MT polymer is determined to be nearly 1 eV from Fig. 4, B and D. The IV curves of the C8OMT polymer (Fig. 4C) are the same as those of I-Au(111) (fig. S4). Thus, the observed HOMO-LUMO gaps of the C8MT polymer as well as the C8OMT polymer on I-Au(111) are substantially lower than those in the solution. There are two possible reasons for these results. Mixing of density of states (DOS) of

the substrate surface (*27*) with DOS of polymers could occur, or there could be charge transfer (*28*) based on the interaction between the polymers and the I-Au(111). These electronic interactions between the polymers and the surface might reduce the HOMO-LUMO gaps compared with those in solution.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S4
References

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Structural Observation of the Primary Isomerization in Vision with Femtosecond-Stimulated Raman

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The primary event that initiates vision is the light-induced 11-cis to all-trans isomerization of retinal in the visual pigment rhodopsin. Despite decades of study with the traditional tools of chemical reaction dynamics, both the timing and nature of the atomic motions that lead to photoproduct production remain unknown. We used femtosecond-stimulated Raman spectroscopy to obtain time-resolved vibrational spectra of the molecular structures formed along the reaction coordinate. The spectral evolution of the vibrational features from 200 femtoseconds to 1 picosecond after photon absorption reveals the temporal sequencing of the geometric changes in the retinal backbone that activate this receptor.

Understanding the mechanism of a chemical reaction requires measuring the structure of the reactant as it evolves into product. Many of the most intriguing and efficient photochemical and photobiological reactions take place on ultrafast time scales and their kinetics have been well characterized by femtosecond absorption and fluorescence spectroscopies (*1*–*5*). Although x-ray diffraction is being developed for time-resolved structural studies of reactions, this approach is challenging to apply in the condensed phase and currently limited to processes slower than ~100 ps (*6*). Ultrafast vibrational spectroscopy is advantageous in this quest because it offers both excellent temporal and structural information (*7*). The traditional picosecond time-resolution limitation (*8*) is being transcended through the use of femtosecond pulses in the infrared (IR) in multidimensional as well as direct time-resolved experiments of ultrafast chemical and biological processes (*9*–*11*). The complementary Raman vibrational techniques have also advanced with the recent development of stimulated Raman in the femtosecond time domain (*12*, *13*), which is valuable because of its ability to interrogate biological processes in aqueous media. Here, we demonstrate the capabilities of femtosecond-stimulated Raman spectroscopy (FSRS) in studies of reaction dynamics by elucidating the molecular mechanism of the primary photochemical events in vision.

In FSRS, two laser pulses drive the Raman transition: a picosecond "Raman pulse" and a femtosecond broadband continuum "probe pulse" that stimulates the scattering of any vibrational modes with frequencies between 600 and 2000 cm^{-1}. The use of the additional probe pulse to induce the Raman scattering offers a number of notable improvements over traditional time-resolved spontaneous Raman spectroscopy (*14*), such as greatly enhanced cross sections and an order-of-magnitude improvement in time resolution (<100 fs) while maintaining excellent energy resolution (<15 cm^{-1}) (*15*, *16*). The impulsive creation of vibrational coherence by the Raman and probe pulses reveals highly time-resolved vibrational structural information that is not accessible by incoherent processes such as spontaneous Raman.

The primary step in vision is the photochemical cis-trans isomerization of the 11-cis retinal chromophore in rhodopsin (Fig. 1A). Production of the primary ground-state transient called photorhodopsin is one of the fastest photochemical reactions in nature and is complete in only 200 fs (*17*). As a consequence, the reaction is extremely efficient, with a quantum yield of 0.65, and about 60% of the incident photon energy is stored in the first thermodynamically stable all-trans retinal photoproduct called bathorhodopsin. This stored energy is then used to drive activating conformational changes in the G protein–coupled receptor that eventually lead to visual sensation. Although a number of theoretical models have been proposed (*18*–*20*) to explain rhodopsin's unique reactive properties, which are responsible for making it an excellent light receptor, many of the most critical questions about its photochemistry remain unanswered. For instance, the coordinates mediating fast excited-state decay, the role of the electronic excited state in the isomerization, the structure of retinal in photorhodopsin, and the nature of the reaction coordinate leading to bathorhodopsin are undefined.

We address these questions by acquiring femtosecond time-resolved vibrational spectra of retinal in rhodopsin throughout the reaction. Modeling of the vibrational structural features after rapid internal conversion to the ground state reveals the highly distorted structure of photorhodopsin. Surprisingly, a large fraction of the atomic rearrangement leading to the formation of fully isomerized bathorhodopsin is shown to occur in the ground electronic state. Vivid details of this

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structural evolution are revealed by the changing frequencies and lineshapes of key vibrational modes during the reaction.

FSRS spectra of rhodopsin after excitation with a 30-fs photochemical pump pulse centered at 500 nm are presented in Fig. 1B (*21*). The reference vibrational spectrum of rhodopsin (bottom) includes the symmetric C–C ethylenic stretch at 1548 cm^{-1}, the fingerprint region from 1100 to 1300 cm^{-1} due to structurally sensitive C–C single-bond stretching and C–H rocking modes, and a feature at 969 cm^{-1} due to concerted hydrogen-out-of-plane (HOOP) wagging motion of the 11 and 12 hydrogens (*22*). The bathorhodopsin reference spectrum at the top of Fig. 1B illustrates the isomerization-induced changes in the intensity and frequency pattern in the fingerprint region as well as the HOOP region; the original mode at 969 cm^{-1} is red shifted and split into three separate peaks at 920, 875, and 850 cm^{-1}, assigned to isolated C_{11}–H, C_{10}–H, and C_{12}–H wagging modes, respectively (*22*).

The fingerprint pattern of photorhodopsin at 200 fs appears to be midway between those of 11-cis rhodopsin and all-trans bathorhodopsin. The features evolve into the bathorhodopsin spectrum over the next 800 fs. In particular, the peak at 1267 cm^{-1} decreases in intensity, whereas the bands at 1216 and 1237 cm^{-1} remain virtually unchanged. Surprisingly, we observed very intense, dispersive lineshapes in the HOOP region between 800 and 950 cm^{-1} at early times. The dispersive HOOP features evolve on the same time scale as the fingerprint bands into the expected three positive features of the bathorhodopsin spectrum. By 1 ps, vibrational cooling has narrowed and blue shifted all features, including the ethylenic stretching band, thereby completing the transformation to bathorhodopsin. These data show that there is considerable reactive evolution on the ground-state surface from 200 fs to 1 ps.

The dispersive lineshapes in the HOOP region at early time delays originate from our capability to monitor structural evolution on the time scale of the reaction in a coherent fashion. This observation can be understood by considering how stimulated Raman signals are generated (*13*). The simultaneous action of picosecond Raman and femtosecond continuum probe pulses drives vibrational coherence in the sample (Fig. 2A). The gating of this process relative to the photochemical pump is temporally well defined by the short (~20-fs) probe pulse. The subsequent coherent vibration of the molecules modulates the macroscopic sample polarization in the time domain, thereby giving rise to positive definite Stokes and anti-Stokes features in the energy domain. Because we detected these features through interference with the unscattered probe, the signal appears on top of the probe envelope. However, if the frequency of this coherent motion initiated by Raman and probe pulses changes during the vibrational dephasing time as shown in Fig. 2B, where the oscillator is chirped from low to high frequency, then the resulting lineshapes become dispersive. The specific lineshape in Fig. 2B, with a negative feature on the high-energy side, is distinctive of a shift from a low- to a high-frequency vibration during the free-induction decay of the vibrational oscillator (*21*). Thus, the dispersive HOOP modes directly report on the dynamic structural relaxation of retinal as it evolves from the high-energy photorhodopsin transient to the ground-state bathorhodopsin product. The heterodyne detection scheme in FSRS is powerful in that it makes possible the observation of vibrational phase and frequency shifts that occur on a time scale shorter than the vibrational dephasing time.

We have simulated the spectral evolution of the HOOP features after internal conversion to the ground state using theory encompassing the above concepts (*21*). The frequencies of three

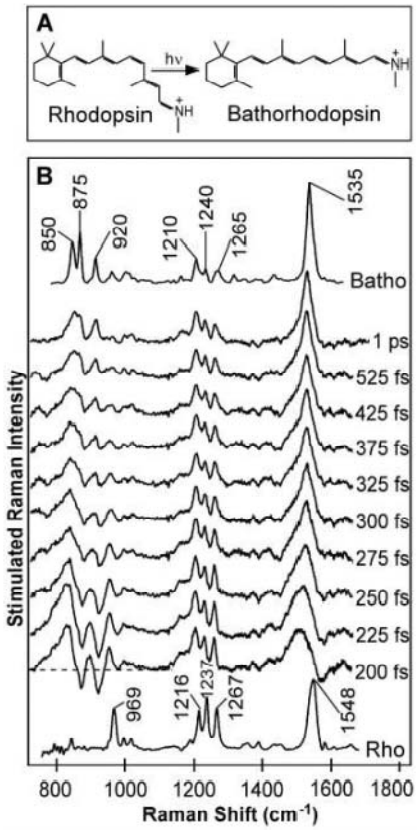

Fig. 1. (**A**) The primary event in vision: 11-cis retinal in rhodopsin is isomerized upon photon (hν) absorption to the all-trans bathorhodopsin photoproduct. (**B**) Time-resolved femtosecond-stimulated Raman spectra of rhodopsin from 200 fs to 1 ps. The spectra are vertically offset and ground state and solvent features as well as a broad sloping baseline have been subtracted (*21*). Resonance Raman spectra of ground-state rhodopsin (Rho) and the trapped bathorhodopsin (Batho) product are included for comparison. The dashed line in the 200-fs plot indicates the spectral baseline.

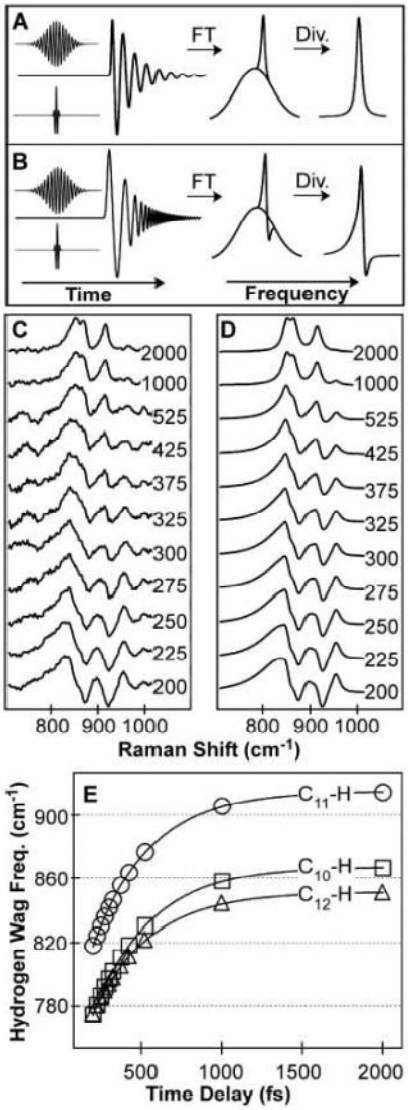

Fig. 2. (**A**) Picosecond Raman and femtosecond probe pulses drive vibrational coherence in the sample. Heterodyne detection yields a gain feature on top of the probe envelope in the energy domain shifted in energy relative to the Raman pulse according to the frequency of the vibration. Division (Div.) of spectra in the presence of the Raman pulse by spectra in its absence results in Lorentzian vibrational features. (**B**) An increase in the vibrational frequency during the vibrational dephasing time gives rise to dispersive lineshapes due to phase-sensitive heterodyne detection of the stimulated Raman signal. FT, Fourier transform. (**C**) Time-resolved stimulated Raman spectra of the HOOP region of reactive rhodopsin from 200 fs to 2 ps. (**D**) Simulated spectra of the HOOP region obtained from a four-mode model using three time-dependent frequencies, as in (B). (**E**) The simulations reveal a ~100 cm^{-1} blue shift of the C_{10}–H (□), C_{11}–H (○), and C_{12}–H (△) frequencies from 200 fs to 2 ps with a ~325-fs time constant.

modes resulting from isolated C_{10}–H, C_{11}–H, and C_{12} H wagging motion were varied exponentially, and a fourth resulting from vibrationally excited but unreactive ground-state species was held constant at 959 cm^{-1}. This simple model is remarkably successful at reproducing the observed spectral dynamics in Fig. 2C. Not only do the simulated spectra duplicate the initially highly dispersive lineshapes, but they also reproduce the temporal evolution of these bands into the traditional bathorhodopsin features (Fig. 2D). The model also yields transient vibrational frequencies of structures along the pathway from photorhodopsin to bathorhodopsin. The HOOP frequencies increase by ~100 cm^{-1} from 200 fs to 2 ps with a ~325-fs time constant (Fig. 2E). Notably, all spectra were simulated with a single set of parameters that are not adjusted to fit the individual time points. The validity of our model is reinforced by the quantitative agreement between experiment and theory in light of the highly constrained parameters used in the simulations.

The evolution of the vibrational structure from 200 fs to 1 ps demonstrates that a large fraction of the net motion along the isomerization coordinate occurs on the ground-state surface. The most marked spectral change on this time scale occurs in the HOOP region where a ~100-cm^{-1} blue shift occurs with a ~325-fs time constant. We recently used vibrational modeling to demonstrate a close relationship between the degree of distortion of the polyene backbone and the anomalously low C_{12}–H wagging frequency in bathorhodopsin (*23*). Specifically, the frequencies of the C_{11}–H and C_{12}–H wagging modes in bathorhodopsin were explained by, at a minimum, the concurrence of same-sense 40° twists about the C_{11}–C_{12} and C_{12}–C_{13} bonds. This previous work suggests that the reduced HOOP frequencies in photorhodopsin are due to even greater distortions of the polyene backbone. The large HOOP frequency increases in the photo-to-batho transition are also physically reasonable because the restoring force for out-of-plane hydrogen wagging motion should increase as the double bonds strengthen in the more planar bathorhodopsin product state.

To test this explanation, we extended the approach of Yan *et al.* (*23*) by using density functional theory (*24*) to calculate the Raman frequencies for intermediate retinal structures that describe the photo-to-batho transition. Structures with calculated wagging frequencies that were in good agreement with the experimental results are presented in Fig. 3. The bathorhodopsin structure is twisted by −144° about the C_{11}=C_{12} and by 31° about the C_{12}–C_{13} bond, consistent with our earlier results, which only considered the position of the C_{12} H wag. Fitting all the wag frequency reductions requires additional twists about adjacent bonds. Bathorhodopsin exhibits intense lines in the Raman spectrum at 850, 875, and 920 cm^{-1} assigned to isolated C_{12}–H, C_{10}–H, and C_{11}–H wagging modes, respectively. Vibrational calculations for the bathorhodopsin structure in Fig. 3C yielded features at 849, 857, and 881 cm^{-1} in excellent agreement with experimental data, except for an underestimated C_{11} H wagging frequency. The photorhodopsin structure is more highly distorted, in particular about the C_9–C_{10} (45°), C_{10}–C_{11} (25°), and C_{11}–C_{12} (−110°) bonds. With these larger twists, the overall shape of retinal is much more like that of 11-*cis* rhodopsin than all-*trans* bathorhodopsin, despite having a formally isomerized (110°) C_{11}=C_{12} bond. Transient frequencies of the isolated C_{10} H, C_{11} H, and C_{12} H wagging modes obtained from the analysis of the 200-fs FSRS spectrum appear at 772, 811, and 762 cm^{-1}, respectively. Calculated frequencies for these modes with the use of the proposed photorhodopsin structure (Fig. 3B) show good agreement with experimental data for the C_{10} H and C_{11}–H modes (814 and 844 cm^{-1}, respectively), although the C_{12}–H frequency is overestimated (853 cm^{-1}). An alternative, but overall similar structure was found in which the C_{10}–H and C_{12}–H frequencies were calculated to drop by ~70 cm^{-1} (835 and 798 cm^{-1}, respectively) with an overestimated C_{11}–H frequency. In general, structures featuring only very specific combinations of backbone twists exhibited large frequency decreases compared with the bathorhodopsin structure.

Although future work certainly requires more detailed calculations that include protein interactions to provide more quantitative modeling of vibrational and energetic data, these results show that large-scale backbone distortions are capable of causing marked frequency drops in all the hydrogen wagging modes. The changes in vibrational structure observed by FSRS are thus best attributed to the dynamic ground-state relaxation of the initially highly twisted photorhodopsin structure as it evolves into bathorhodopsin.

Taken together, the data and modeling presented here are consistent with the following overall picture of the light-induced retinal isomerization that initiates vision (Fig. 4): The reaction begins with rapid excited-state decay after Franck-Condon excitation. Because optical excitation is strongly allowed, the transition from the A_g ground electronic state must populate an excited state having ungerade symmetry. Thus, any nuclear distortion that efficiently couples the Franck-Condon state to the ground state resulting in fast excited-state decay must be nontotally symmetric, such as the A_2 HOOP or backbone torsions. Critically, the isomerization can also only occur along nontotally symmetric coor-

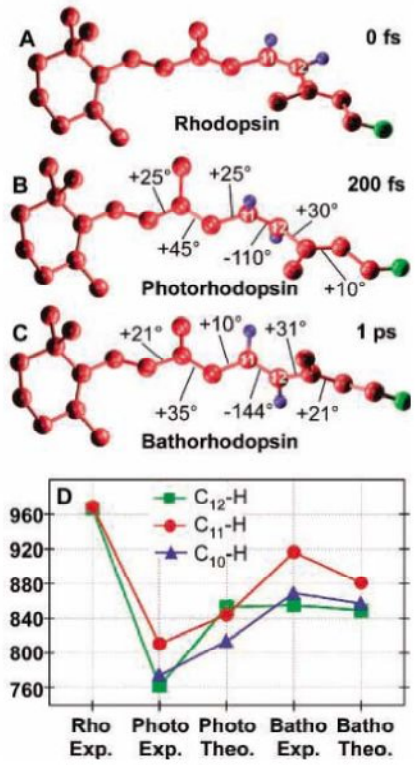

Fig. 3. (A to C) Retinal chromophore structures for reactant rhodopsin (*28*) and for photorhodopsin and bathorhodopsin that reproduce the observed hydrogen wagging frequencies. Backbone dihedral twist angles from the rhodopsin reactant are indicated. (D) Comparison of density functional theory (*24*) calculated (Theo.) and experimental (Exp.) hydrogen wagging frequencies for the photo and bathorhodopsin structures.

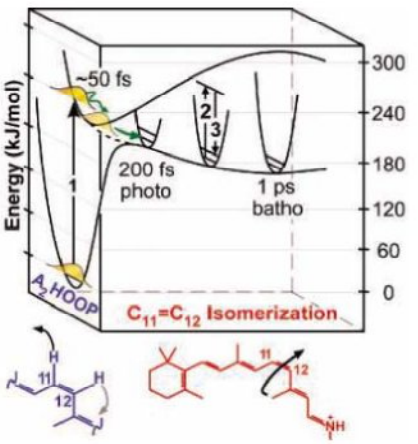

Fig. 4. Multidimensional representation of the isomerization coordinate for the primary event in vision. Absorption of a visible photon is followed by rapid motion out of the Franck-Condon region along high-frequency HOOP coordinates (vibrational period ~36 fs) which carry the system toward a conical intersection in ~50 fs. Curve crossing to the ground state to form highly distorted photorhodopsin is complete by ~200 fs. The structural evolution of retinal on the ground-state surface along the C_{11}–C_{12} torsional as well as other coordinates produces all-*trans* bathorhodopsin in ~1 ps. Also shown is the energy level diagram for coherent femtosecond-stimulated Raman probing of the ground-state molecular dynamics where (1) is a femtosecond photochemical pump pulse, (2) is a narrow bandwidth Raman pulse, and (3) is a broadband femtosecond probe.

dinates. The extremely short lifetime of the excited state (~50 fs), as established by transient absorption (*17*), resonance Raman intensity analysis (*25*), and spontaneous fluorescence measurements (*26*), however, severely restricts the extent of atomic displacements that can occur on this time scale. Given the energy available to the chromophore, the maximum C_{11}–C_{12} dihedral angle that can be achieved in 50 fs is ~50°, even if restrictions from the protein pocket are ignored (*25*, *27*). This suggests that the role of C_{11}–C_{12} torsional motion during the excited-state lifetime is limited. The similarity of the vibrational period of the 969 cm^{-1} $C_{11}H$=$C_{12}H$ HOOP (~36 fs) to the excited-state lifetime (~50 fs) supports its role in facilitating internal conversion. Additionally, resonance Raman intensity analysis shows quantitatively that retinal undergoes rapid distortion along the $C_{11}H$–$C_{12}H$ HOOP coordinate after optical excitation as a consequence of the lowered overall symmetry of the molecule when bound to rhodopsin (*25*). We thus conclude that excited-state decay through a conical intersection is mediated largely by fast HOOP motion.

Evolution along the C_{11}=C_{12} torsional coordinate after internal conversion leads to the formation of photorhodopsin with a formally isomerized (>90°) C_{11}–C_{12} bond but an overall highly distorted structure. Adjacent single- and double-bond twists compensate for the local cis-trans isomerization resulting in an overall reactant-like shape that, although isomerized about the C_{11}=C_{12} bond, minimizes steric interactions with the protein pocket, thereby enabling the fast reaction rate (compare Fig. 3, A and B). The molecule then uses the ~5000 cm^{-1} of energy available from rapid barrierless internal conversion as well as the ~3000 cm^{-1} from the photo-to-batho relaxation to drive the larger scale structural changes necessary to form the all-trans bathorhodopsin photoproduct in ~1 ps (Fig. 3C). Thus, although the isomerization is initiated in the excited state and photorhodopsin is formally trans about the C_{11}–C_{12} bond, much of the geometric changes associated with the isomerization actually occur on the ground potential surface in the photo-to-batho transition. This result is a direct consequence of the different time scales for complete excited-state decay (~200 fs) and bathorhodopsin formation (~1 ps) determined in this work.

This multidimensional model for rhodopsin isomerization, including a fast "gating" coordinate (HOOP), deviates substantially from the one-dimensional picture commonly used to describe photoisomerization reactions, where both electronic and nuclear dynamics occur along the same, slow torsional coordinate. Furthermore, these observations make it possible to better understand the role of the protein in determining rhodopsin's unique reactivity. The tight binding pocket influences the reaction path in three ways: (i) It primes the molecule for rapid excited-state decay along the HOOP coordinate by pretwisting the retinal backbone, (ii) it restricts the possible motion of the excited chromophore through steric interactions with surrounding amino acids, thereby promoting reaction speed and resulting in a high isomerization quantum yield, and (iii) it captures the high-energy bathorhodopsin product and efficiently transfers this energy into protein conformational changes that activate the receptor. We anticipate that these concepts will be important in understanding many efficient photobiological reactions.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S3
Table S1

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The Mid-Pleistocene Transition in the Tropical Pacific

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A sea surface temperature (SST) record based on planktonic foraminiferal magnesium/calcium ratios from a site in the western equatorial Pacific warm pool reveals that glacial-interglacial oscillations in SST shifted from a period of 41,000 to 100,000 years at the mid-Pleistocene transition, 950,000 years before the present. SST changes at both periodicities were synchronous with eastern Pacific cold-tongue SSTs but preceded changes in continental ice volume. The timing and nature of tropical Pacific SST changes over the mid-Pleistocene transition implicate a shift in the periodicity of radiative forcing by atmospheric carbon dioxide as the cause of the switch in climate periodicities at this time.

In the mid-Pleistocene, ~950 thousand years (ky) before the present (B.P.), the climate of Earth underwent profound changes in the length and intensity of its glacial cycles. This mid-Pleistocene transition (MPT), as indicated by benthic foraminiferal $\delta^{18}O$, was characterized by a change in the dominant periodicity of high-latitude climate oscillations from 41 ky to 100 ky; a positive shift in mean benthic $\delta^{18}O$, generally ascribed to continental ice-sheet expansion; and an increase in the amplitude variability of $\delta^{18}O$, attributed to more severe glaciations after 950 ky B.P. (*1*–*3*). Most of the hypotheses offered to explain these changes involve high-latitude Northern Hemisphere processes such as ice-sheet or sea-ice dynamics (*2*, *4*, *5*). Recent paleoclimatic reconstructions, however, have shown that during the MPT, the tropics also experienced major changes that resemble some aspects of high-latitude climate variability but also have their own unique patterns (*6*, *7*). Current hypotheses cannot fully explain these observations and the common

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characteristics revealed by paleoclimatic reconstructions from low and high latitudes.

To test hypothesized causes for the mid-Pleistocene transition, we reconstructed detailed thermal and $\delta^{18}O$-seawater histories spanning the MPT from a site in the heart of the western equatorial Pacific (WEP) warm pool (Fig. 1). This location is ideal for testing hypotheses that address proposed forcing mechanisms of tropical climate variability because (i) warm-pool thermal variability is linked throughout the tropics by convection (*8*); (ii) the warm pool is less subject to regional oceanographic influences such as thermocline depth changes, as demonstrated by the small response of warm-pool sea surface temperatures to El Niño/Southern Oscillation (ENSO) variations (Fig. 1) (*9*); and (iii) the warm pool is remote from the direct radiative influence of continental ice sheets and has the most direct response to radiative forcing as a result of changes in atmospheric greenhouse gases (*10*, *11*).

We determined sea surface temperatures (SSTs), $\delta^{18}O_c$ and $\delta^{18}O$-seawater ($\delta^{18}O_w$) from Ocean Drilling Program (ODP) Hole 806B (0°19.1′N, 159°21.7′E, 2520-m water depth) (*12*) on the Ontong Java Plateau, using the surface-dwelling planktonic foraminifer *Globigerinoides ruber* (Fig. 2). Our records reach back to 1.3 million years (My) B.P., with an average resolution of 2.3 ky, extending a previous study (*13*). We used the Mg-paleothermometry technique, which is based on the temperature dependence of Mg substitution in calcite, and calculated $\delta^{18}O_w$ following previous protocols (*13*, *14*). We constructed the Hole 806B age model by visual alignment of the *G. ruber* $\delta^{18}O$ sequence to the ODP Hole 677 benthic $\delta^{18}O$ record (*14*, *15*). Hole 806B has remarkably constant sedimentation rates (2.0 ± 0.3 cm/ky) from 0.45 to 1.3 My B.P. and, because of its location above the present-day lysocline depth, it also has good preservation of foraminifer shells. There are only two coring gaps of ~0.9 m (~50 ky) that include parts of marine isotope stage (MIS) 19 and MIS 37 (*14*).

The *G. ruber* $\delta^{18}O$ data indicate 12 glacial-interglacial (G-I) oscillations from MIS 13 to 41 between 450 and 1348 ky B.P., in agreement with reference foraminiferal $\delta^{18}O$ records (*12*, *15*) (Figs. 2 and 3). Over the past 900 ky, the G-I range of $\delta^{18}O$ is larger by about one-third than the corresponding early Pleistocene (900 to 1348 ky) range, but mean $\delta^{18}O$ remains the same [–1.60 ‰ (per mil) and –1.56 ‰, respectively]. Spectral analysis of the Hole 806B $\delta^{18}O$ data indicates that over the past 900 ky, the 100-ky period component explains more than 70% of the variance in $\delta^{18}O$, whereas during the early Pleistocene (EP) similar power is shared by ~90-ky and 41-ky–related periodicities, with a minor contribution from the 23-ky period. The presence of significant power at a ~90-ky period might be the result of a strong salinity component at site 806B during the early Pleistocene (*16*).

The observed Mg/Ca-derived SST average from the early and mid-Pleistocene time intervals combined (from 500 to 1348 ky B.P.) is 27.8°C, similar to the late Pleistocene (0 to 500 ky B.P.) SST average of 27.4°C (*13*). The G-I SST range over the mid-to-early Pleistocene is smaller than that during the late Pleistocene, 3°C versus 4.3°C, respectively. This difference is largely a consequence of warmer (~0.7°C) glacial intervals, relative to the late Pleistocene; average interglacial SSTs (~29°C) are similar throughout the record. The warmest temperatures during

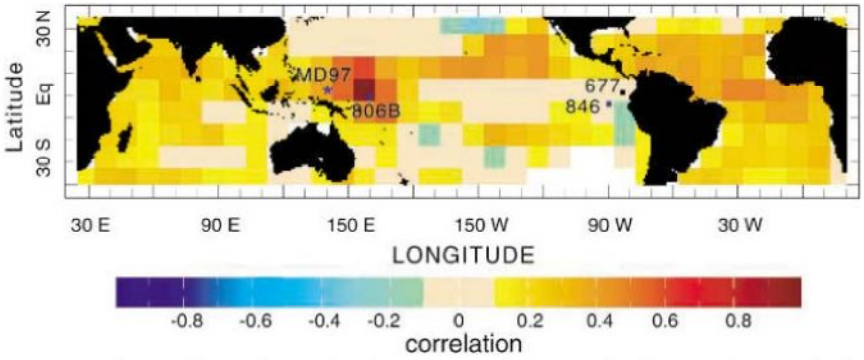

Fig. 1. Map showing the correlation of Kaplan interannual SST anomalies between the site of Hole 806B (0°19.1′N, 159°21.7′E) and other regions of the tropics (*28*). Correlations are based on SST data on a 5 by 5 grid of monthly anomalies from 1856 to 2003. The base period used for the anomalies is 1951 to 1980. Locations of ODP Hole 846 (*6*) (3°5′S, 90°49′W) and MD97-2140 (*7*) (2°02′N, 141°46′E) are also indicated. Warm-pool SST anomalies near Hole 806B are positively correlated with temperature anomalies in a wide swath of the tropical oceans. Warm-pool anomalies are either uncorrelated or are anticorrelated with anomalies in the eastern equatorial Pacific cold tongue (i.e., Hole 846), a consequence of their opposite behavior during ENSO changes (*20*). The location of ODP Hole 677 [reference benthic foraminiferal $\delta^{18}O$ record in (*15*)] is also shown (1°12′N, 83°44′W).

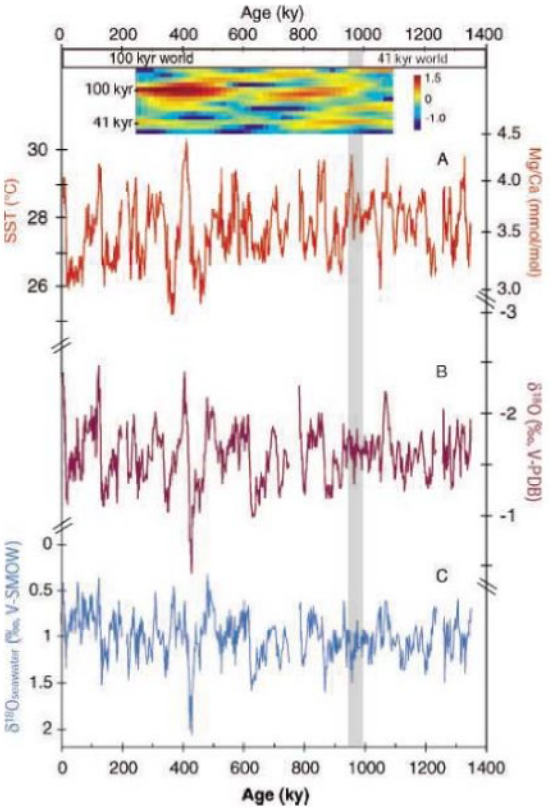

Fig. 2. Western equatorial Pacific ODP Hole 806B records based on the surface-dwelling foraminifera *G. ruber*. This record includes a previous (*13*) reconstruction for the late Pleistocene (4 to 470 ky B.P.). Gaps in the records are the result of coring gaps. The chronology is based on wiggle matching to the target core ODP Hole 677 benthic foraminiferal $\delta^{18}O$ (*14*, *15*). (**A**) *G. ruber* Mg/Ca-derived SST record. Mg/Ca data were converted using the relationship: SST (C°) = 0.089^{-1}*ln[Mg/Ca (m) / 0.3] (*13*). Each point is the average of two to four replicates. The mid-Pleistocene transition (MPT) at ~950 ky B.P. is indicated by a gray bar. Evolutionary spectral analysis plot for SST (top) from 250 to 1100 ky B.P. is based on the multitaper method (*14*). The scale represents Log_{10} units^2 per cycle per ky. The evolutionary spectral analysis reveals that the SST record is dominated by the ~100-ky period over the last 950 ky B.P. and by ~41-ky and ~60-ky–related periods from 1346 ky to 950 ky B.P. (**B**) *G. ruber* $\delta^{18}O$ record. The G-I range of $\delta^{18}O$ increases over the MPT. (**C**) $\delta^{18}O$-seawater record, calculated by extracting the component in planktonic $\delta^{18}O$ explained by the Mg/Ca-derived SST using the low-light paleotemperature equation determined for *Orbulina universa* (*29*). Neither the calcite nor the seawater $\delta^{18}O$ records shows a positive shift over the MPT.

the mid- and early Pleistocene occurred in MIS 25 (29.8°C), 952 ky B.P., and the coldest in MIS 30 (26°C), 1052 ky B.P. (Fig. 2). The mid-Pleistocene transition is well represented in the Hole 806B SST record (Fig. 2). Mean SST and the average G-I change in SST do not shift over the MPT, in contrast to changes observed in benthic $\delta^{18}O$ (Figs. 3 and 4). Average SSTs during the early and mid-Pleistocene (500 to 900 ky B.P.) are virtually identical, 27.9 ± 0.7°C and 27.7 ± 0.7°C (1 SD), respectively (Fig. 2).

The G-I range in $\delta^{18}O_w$, calculated from measured $\delta^{18}O$ and inferred temperatures, is ~0.7‰ over the full length of the record (Fig. 2). A previous study of the late Pleistocene record from Hole 806B (*13*) demonstrates that $\delta^{18}O_w$ at this site is strongly influenced by hydrological changes on G-I time scales. In addition to orbital frequencies, the $\delta^{18}O_w$ time series also shows quasiperiodic ~200-ky cycles during the early and mid-Pleistocene time interval. These cycles might be related to long-term hydrological evolution in this region, suggesting that $\delta^{18}O_w$ is not a simple proxy of ice volume at this site. The G-I range of Hole 806B $\delta^{18}O_w$ increases by ~0.16‰ during the MPT, from an early Pleistocene value of 0.72‰ to a mid-Pleistocene value of 0.88‰, which likely reflects increasing variability in continental ice as suggested by benthic foraminiferal records (*1*–*3*).

The Hole 806B SST record is spectrally similar to the ODP Hole 677 reference benthic foraminiferal $\delta^{18}O$ record (*15*), with a characteristic dominance of ~100-ky and 41-ky periods and a much weaker contribution at 23 ky (Figs. 2 and 3). As suggested by evolutionary spectral analysis of Hole 806B SST and Hole 677 benthic foraminiferal $\delta^{18}O$, the transition between the 41-ky and 100-ky–dominant modes of variability occurred at ~950 ky B.P. (Fig. 4, right panels). Point-to-point comparison between these two records over the MPT reveals that *G. ruber* SST leads benthic $\delta^{18}O$ by ~3 ky (Fig. 4). Furthermore, cross-spectral analysis between *G. ruber* SST and benthic foraminiferal $\delta^{18}O$ reveals that SST leads benthic $\delta^{18}O$ by 3 ± 1.2 ky [95% confidence interval (CI)] at the 41-ky–dominant period during the early Pleistocene.

SST records from two other sites in the tropical Pacific, one in the eastern equatorial cold tongue (*6*) and a second in the area of strong intertropical convergence zone influence northwest of our site (*7*), provide basinwide context for our records (Fig. 1). Comparison of these SST records from 1348 to 900 ky B.P. suggests a strengthening of the zonal equatorial Pacific SST gradient by ~1.3°C, due almost entirely to the cooling in the eastern Pacific. The development of this SST gradient occurred during a time interval in which there was no secular change in WEP SSTs, as revealed by the two western Pacific SST records (*7*) (Fig. 3). High-latitude climate, as indicated by the Hole 677 $\delta^{18}O$ record, was also relatively stable at this time (Fig. 3). Statistical analysis of benthic foraminiferal records and the Hole 806B $\delta^{18}O_w$ series reveal that high-latitude climate was relatively stable for more than 400 ky before the MPT (Fig. 3) (*17*). This observation suggests that the intensification of tropical Pacific zonal temperature gradients and the inferred enhancement of the Walker circulation at this time was not accompanied by regional long-term hydrological changes in the WEP and Northern Hemisphere high-latitude climate reorganizations, in contrast to previous suggestions (*7*, *18*).

The long-term surface cooling of the eastern-boundary upwelling regions from 1350 to 900 ky

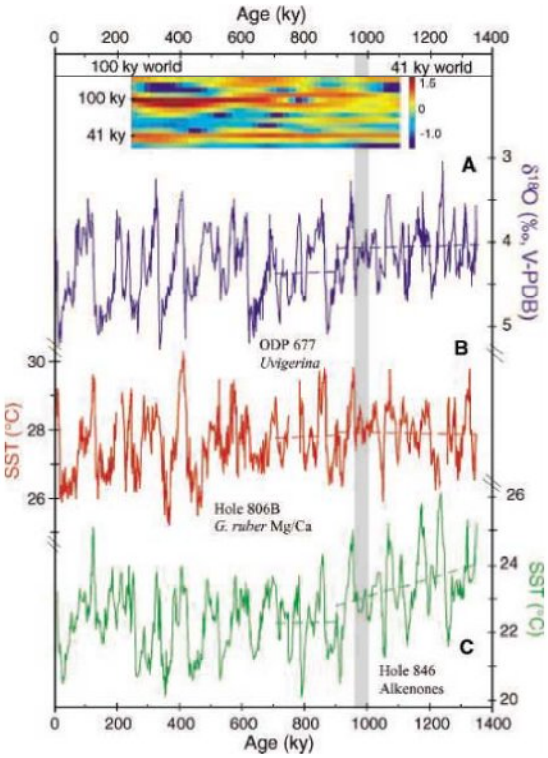

Fig. 3. (**A**) ODP Hole 677 benthic foraminiferal $\delta^{18}O$ record (*15*) showing a mean positive shift of 0.25‰ at ~900 ky B.P. and long-term stability from 1340 to 900 ky B.P. (dashed lines are linear regressions). The linear regression of $\delta^{18}O$ over this time interval is not statistically significant (*17*). Top: Spectrogram of benthic $\delta^{18}O$ from 250 to 1100 ky B.P. showing the shift in the dominant period from 100 ky to 41 ky at ~950 ky B.P. (gray bar) (*14*). The scale represents Log_{10} units^2 per cycle per ky. (**B**) Hole 806B *G. ruber* SST record showing no significant secular change from 1340 to 800 ky B.P. and long-term thermal stability over the MPT (red line before the transition represents a nonsignificant linear regression; slope = zero, 95% CI). (**C**) SST record from eastern equatorial Pacific ODP Hole 846 (3°5′S, 90°49′W) based on the alkenone unsaturation index (*6*). The statistically significant linear regression (slope ≠ zero, 95% CI) showing a secular cooling trend from 1340 to 900 ky B.P. is indicated (green line). As a consequence, from 1340 to 900 ky the equatorial Pacific temperature zonal gradient increased by ~1.3°C.

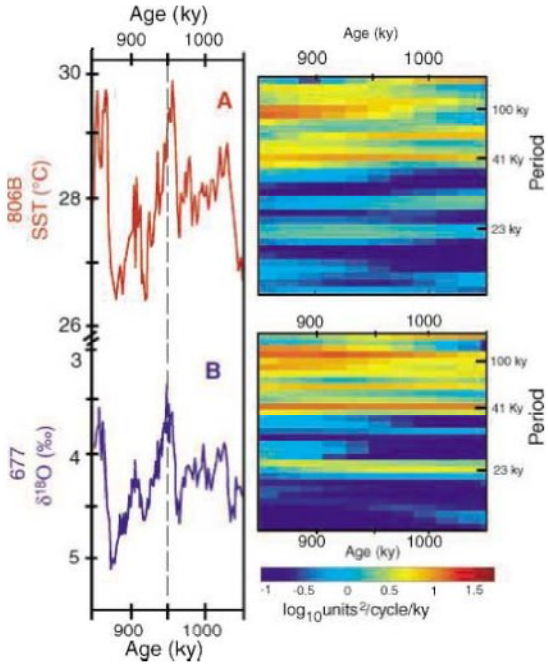

Fig. 4. Blowup of the mid-Pleistocene transition as seen in the Hole 806B SST record (**A**) and the Hole 677 benthic foraminiferal $\delta^{18}O$ record (**B**). Right panels show a blowup over the MPT of the spectrograms shown in Figs. 2 and 3. The interglacial peak centered on 950 ky B.P. is MIS 25. Note the presence of the positive shift in benthic $\delta^{18}O$ at ~900 ky B.P. and the absence of a similar shift in SST at that time. The transition between the 41-ky and 100-ky variability occurs between 950 and 1000 ky B.P. in both records. Point-to-point comparisons between the two signals suggest that SSTs lead benthic $\delta^{18}O$ by ~3 ky over the MPT. The same pattern can be seen between SST and planktonic $\delta^{18}O$ in Hole 806B (Fig. 2).

B.P. (*6*), while the warm pool and Northern Hemisphere climate remained relatively stable, may be related to secular changes in the density of the deep ocean that influenced the depth of the thermocline, as previously predicted (*9*). This cooling has been invoked by a number of hypotheses addressing the MPT (*7*, *18*). The thermal stability of the WEP, where SSTs are expected to respond thermodynamically to atmospheric radiative forcing, and the coinciding high-latitude climate stability from 900 to 1350 ky B.P. reflected by benthic $\delta^{18}O$ records, do not support changes in radiative forcing as the cause of the inferred eastern equatorial Pacific (EEP) secular cooling trend (*7*). Benthic foraminiferal carbon isotopic records, interpreted as a proxy of the thermohaline circulation, show a decrease in the $\delta^{13}C$ contrast between the North Atlantic and Pacific from 1.3 My B.P. to ~800 ky B.P. (*19*). The cooling in the EEP might reflect shallowing of the thermocline resulting from an increase in stratification produced by the deep-ocean circulation rearrangements suggested by $\delta^{13}C$ records. Model calculations suggest that a modest change in the temperature difference across the thermocline of only a few tenths of a degree can produce changes in the EEP SSTs of over 1°C (*9*).

The spectral properties of the Hole 806B SST record provide a powerful test of current hypotheses addressing Pleistocene tropical and high-latitude climate variability and the mid-Pleistocene transition. The spectral resemblance between the WEP (Hole 806B) and EEP (Hole 846) (*6*) SST records is striking (Fig. 3). Sea surface temperature variations in both end members of the equatorial Pacific are statistically coherent and in phase within the 2-ky resolution of the sites, and both records switch from 41-ky to 100-ky–dominant periods during the MPT (fig. S3). Furthermore, the early Pleistocene G-I SST range from both Hole 806B and Hole 846 is similar, 3°C and 4°C, respectively. Today, SSTs in the EEP are strongly influenced by wind-driven thermocline depth changes (*20*). In the WEP, where the thermocline is very deep (>100 m), SSTs are much less likely to be affected by thermocline depth changes (*9*, *20*). Because of this difference, interannual SST anomalies in the EEP cold tongue associated with the ENSO phenomenon are not correlated with anomalies near site 806B (Fig. 1). Further support for differences in the thermal evolution of the two equatorial Pacific end members lies in the observed long-term thermal stability of the WEP during the interval in which the EEP became progressively colder, intensifying Pacific zonal gradients after 1350 ky B.P. (Fig. 3). These observations suggest that a mechanism that invokes changes in thermocline depth (*6*) is unlikely to explain the observed warm-pool SST variability, because such a mechanism would not produce strong 41-ky cycles in SST in the WEP. On the other hand, as pointed out by Lin and Herbert (*6*), the sense of annual insolation changes in the tropics as driven by obliquity variations is in the opposite direction of that required to drive the observed tropical SST changes. We suggest instead that both end members of the equatorial Pacific responded to a common factor: atmospheric CO_2 forcing.

Consideration of the radiative forcing by different components potentially implicated in the Last Glacial Maximum suggests that atmospheric CO_2 changes are the dominant source of radiative forcing in the tropical ocean regions (*10*). A crucial role of atmospheric CO_2 in forcing tropical and Southern Hemisphere climate variability is strongly suggested by the observation that Antarctic air temperatures (*21*, *22*), tropical SSTs (*11*), and bottom-water temperatures (*23*) are in phase with atmospheric CO_2 and lead benthic foraminiferal $\delta^{18}O$ by several thousand years during the late Pleistocene. In the same manner, spectral comparisons between tropical SST records from the three sites in the tropical Pacific and foraminiferal $\delta^{18}O$ over the early Pleistocene reveal that all three SST records lead foraminiferal $\delta^{18}O$ by 3 to 7 ky at the dominant 41-ky period (table S3). The inferred lead of SST over continental ice volume rules out the hypothesis that tropical SST variability is controlled by the direct radiative influence of Northern Hemisphere continental ice sheets. The observed pattern of early and mid-Pleistocene tropical climate variability, marked by synchronous and similar magnitude SST cycles in both the warm and cold end members of the tropical Pacific, and with a clear lead of both over continental ice volume changes, is remarkably similar to late Pleistocene climate observations (*11*). The character of Pleistocene climate evolution suggests that the shift in tropical climate variability from a 41-ky to a 100-ky–dominated system (Figs. 3 and 4) is the result of changes in greenhouse forcing as mediated by the radiative effect caused by variability in atmospheric CO_2. We speculate that the global carbon system, acting as an internal self-sustained oscillator sensu (*4*), was paced by obliquity changes during the early Pleistocene (*24*): this response shifted to the eccentricity envelope of precession after the mid-Pleistocene transition. Future reconstructions of atmospheric CO_2 extending back to the MPT, projected as part of the European Project for Ice Coring in Antarctica (EPICA) (*22*, *25*), would be a direct test of this hypothesis.

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17. To evaluate the presence of statistically significant long-term trends in benthic $\delta^{18}O$ records from 900 ky to 1346 ky B.P., we performed linear regressions and tested the null hypothesis of zero slope (95% CI) from ODP Holes 677 (*15*), 846 (*26*), and 849 (*19*). These results indicate that there is no statistically significant long-term trend in benthic foraminiferal $\delta^{18}O$ in any of the three records. The lack of long-term trends in benthic foraminiferal $\delta^{18}O$ records at this time interval has been previously detected using independent statistical tools (*27*).
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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 to S3
References

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Recent Ice-Sheet Growth in the Interior of Greenland

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A continuous data set of Greenland Ice Sheet altimeter height from European Remote Sensing satellites (ERS-1 and ERS-2), 1992 to 2003, has been analyzed. An increase of 6.4 ± 0.2 centimeters per year (cm/year) is found in the vast interior areas above 1500 meters, in contrast to previous reports of high-elevation balance. Below 1500 meters, the elevation-change rate is –2.0 ± 0.9 cm/year, in qualitative agreement with reported thinning in the ice-sheet margins. Averaged over the study area, the increase is 5.4 ± 0.2 cm/year, or ~60 cm over 11 years, or ~54 cm when corrected for isostatic uplift. Winter elevation changes are shown to be linked to the North Atlantic Oscillation.

The Greenland Ice Sheet is an object of increased attention for at least two reasons related to global climate change (*1*, *2*). First, complete melting of the ice sheet would raise the global sea level up to 7 m. This process, expected to occur on a millennial time scale, should begin when the critical ~3°C threshold for Greenland climate warming is crossed, perhaps before the end of this century (*2*, *3*). Second, increased Greenland Ice Sheet melt and freshwater input into the northern North Atlantic Ocean have been theorized to weaken or even disrupt the global thermohaline circulation on a relatively rapid, multidecadal time scale (*4*, *5*). Here, we address changes in the surface elevation of the interior of the Greenland Ice Sheet, which is pertinent to both of these critical issues through glacier mass balance, i.e., accumulation minus losses.

The response of the Greenland Ice Sheet to climate forcing is not straightforward, because variability in solar radiation, greenhouse gases (GHGs), atmospheric circulation, surface temperature, cloud cover, precipitation, and albedo, as well as glacier-flow dynamics, may affect the magnitude, rate, and direction of changes in glacier mass balance (*1*–*3*, *6*). Efforts to measure changes in the Greenland Ice Sheet from field observations and aerial and satellite remote sensors have improved our knowledge over the past decade, although there is as yet no consensus assessment of the overall mass balance of the ice sheet (*6*). There is nonetheless considerable evidence of melting (*7*–*9*) and thinning (*10*, *11*) in the coastal marginal areas in recent years, as well as indications that large Greenland outlet glaciers can surge at subdecadal time scales (*12*), possibly in response to climate. Less known are changes that may be occurring in the vast elevated interior area of the ice sheet, although a balance has been reported based on some tracks of aerial laser altimetry, unevenly sampled in space and time (*10*, *13*). This underscores the need for long, continuously sampled data sets, such as those derived from satellite altimetry. Whereas decadal and longer satellite-derived data sets have been developed for surface melt (*7*–*9*), the surface-elevation data sets analyzed previously have been discontinuous (*10*, *11*, *13*) and relatively short (*14*).

Therefore, we derive and analyze a continuous satellite altimeter height record of Greenland Ice Sheet elevations by combining European Space Agency (ESA) ERS-1 and ERS-2 data to (i) determine the spatial patterns of surface elevation changes over an 11-year period, 1992 to 2003, (ii) determine seasonal and interannual variability of the surface elevation over the same period, and (iii) investigate how observed elevation changes are linked to the North Atlantic Oscillation (NAO) pattern of atmospheric circulation (*15*), which we hypothesize to have an underappreciated role on the Greenland Ice Sheet surface elevation through its effect on winter precipitation. This is a critical issue, as the NAO index (*16*) is predicted to become more positive in response to increasing GHGs (*17*, *18*).

The data set analyzed here to identify Greenland Ice Sheet surface-elevation changes is based on 11 consecutive years of ERS-1 and ERS-2 radar altimeter height measurements (*19*). The methodology used to calculate elevation changes is based on the crossover analysis using the differences in ice-mode altimeter heights at crossing points of the satellite-orbit ground tracks (*19*). Elevation change rates (dH/dt) were calculated for 0.5° latitude × 1.0° longitude cells using two methods. In the first method, the dH/dt method (*20*), we used all available crossovers. The dH/dt was determined as a slope of a linear fit to the crossover difference of elevations versus time interval using descending minus ascending orbits. The second method, the time series method (*21*)—was applied to form seasonally averaged time series of elevation change, using descending minus ascending orbits and ascending minus descending orbits (*19*). Thus, the first method gives the spatial elevation change averaged for the entire time interval, whereas the second method allows investigation of the temporal variability of spatial averages.

However, to merge ERS-1 and ERS-2 as one data set, it is essential to account for bias between the satellites. To achieve this, we developed and applied the following procedures. We applied the systematic 40.9-cm offset, with ERS-2 being lower than ERS-1, specified by ESA (*22*) and confirmed by Brenner and colleagues (*23*), before investigating the remaining bias. Although there was a year (1995 to 1996) when the satellites operated in tandem, the number of ERS-1/ERS-2 crossover points available during this period is considered insufficient to determine the between-satellite bias directly from elevation differences during the overlap (*19*). Therefore, we estimated the bias using a large number (8 mil-

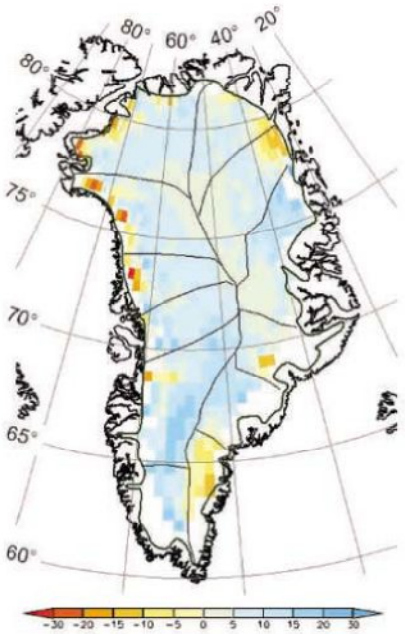

Fig. 1. Greenland, showing the boundaries (thick line) of the ice sheet and major ice divides (thin lines), adapted from (*13*). The colors indicate ice-sheet elevation change rate (dH/dt) in cm/year, derived from 11 years of ERS-1/ERS-2 satellite altimeter data, 1992 to 2003, excluding some ice-sheet marginal areas (white). The spatially averaged rate is 5.4 ± 0.2 cm/year, or ~5 cm/year when corrected for isostatic uplift. The white areas between the color-coded pixels and the thick line delimiting the ice sheet indicate no observations. Latitude in °N, longitude in °W.

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lion) of crossover points between ERS-1 orbits during its whole period of operation from 1992 to 1996 and ERS-2 orbits from a period of equal length, 1995 to 1999, including the 1-year overlap, giving higher reliability (*19*) (fig. S1). The calculated spatially averaged ERS-1/ERS-2 bias is 21.5 ± 2.0 cm. The bias is spatially variable, and the effect of the bias on determining *dH/dt* from the crossover data varies from typically ~2 cm/year over the interior plateau to about 20 cm/year over ice-sheet margins (*19*). We applied this bias for each ERS-1 × ERS-2 crossover point before calculating the *dH/dt* average for each cell.

The spatial pattern of variability derived from the *dH/dt* method is mapped as the 11-year elevation-change rate for each cell (Fig. 1), based on 45 million crossover points distributed over three data sets: ERS-1 (ERS-1 × ERS-1), ERS-2 (ERS-2 × ERS-2), and ERS-1 and ERS-2 (ERS-1 × ERS-2). Positive *dH/dt* values are generally found over most of the high-elevation areas, with largest positive values of up to 10 to 20 cm/year in southwestern (<69°N) and eastern Greenland between 74°N and 77°N. The largest negative values, –25 to –30 cm/year, are found in several parts of western Greenland, where independent aerial altimetry in 1997 and 2002 to 2003 also found the greatest thinning (*11*). Negative values are also found in southeastern Greenland (63°N to 66°N) and in the northeastern ice stream (78°N to 80°N), with values of –10 to –15 cm/year. The regional differences in elevation change reflect, to varying degrees, the location of ice divides (Fig. 1), notably between southwest and southeast Greenland, +10 to +20 cm/year and –5 to –15 cm/year, respectively. The most substantial thinning is observed over outlet glacier areas, particularly in western, southeastern, and northeastern Greenland, which implies a dynamic mechanism in addition to changes in precipitation and melting [e.g. (*24*, *25*)].

The surface-elevation change rate averaged over the Greenland Ice Sheet [excluding those marginal cells with unreliable data (*19*)] is +5.4 ± 0.2 cm/year, or ~60 cm for the period 1992 to 2003. We have partitioned the variability into different elevation bands of 500-m intervals, starting at <1500 m and extending to >3000 m (Table 1). Below 1500 m, where summer melting is pronounced, the mean *dH/dt* is 2.0 + 0.9 cm/year for the period 1992 to 2003. Above 1500 m, the mean *dH/dt* is 6.4 + 0.2 cm/year. These *dH/dt* values are obtained before correcting for isostatic uplift, which is estimated to be approximately 0.5 cm/year averaged for the entire Greenland Ice Sheet (*26*). When adjusted for average uplift, the overall ice thickness changes are thus about 5 cm/year or 54 cm over 11 years, whereas above 1500 m, these values are about 6 cm/year or 65 cm over 11 years. The latter results are in contrast to the high-elevation balance reported previously (*10*, *13*), based on spatially and temporally discontinuous observations, in contrast to our 11-year data set comprising 45 million crossover points. The positive changes observed here imply increased accumulation, supported by evidence that elevation changes in the interior of Greenland can be attributed primarily to snow accumulation (*27*).

The time-series analysis (*19*) of elevation changes spatially averaged over all cells <1500 m and >1500 m indicates seasonal and interannual variability of up to tens of cm (Fig. 2). Below 1500 m, there is no significant trend until 1999, after which a negative trend of ~6 cm/year is evident. Above 1500 m, the positive change is 6.1 ± 0.6 cm/year, confirming the result from the *dH/dt* method. The overall elevation change derived from the time-series method is +5.3 ± 0.5 cm/year, also confirming the *dH/dt* result.

Regional temperature and precipitation are both influenced by the NAO (*15*). Because the NAO in winter strongly affects precipitation, with $r \sim 0.75$ for model-calculated total precipitation for Greenland and $r \sim -0.80$ for southern Greenland (*28*), we hypothesized that the NAO weather and precipitation pattern

Table 1. Spatially averaged elevation-change rates (*dH/dt*) and SE partitioned over different elevation bands of the Greenland Ice Sheet, 1992 to 2003, not corrected for isostatic uplift. The uncertainties (±) in columns 2 and 3 are SE when averaging results within each band. The values in column 3 are SE of the slope of the linear fit determined for each cell. The areas corresponding to each elevation band are indicated in column 4. These values exclude those cells with unreliable, discarded data (Fig. 1) (*19*), mostly from the lowest elevation band.

Elevation band (km)	*DH/dt* (cm/year)	Standard error (cm/year)	Area ($10^3 \times km^2$)
<1.5	–2.0 ± 0.9	0.4 ± 0.04	155.1
1.5–2	5.6 + 0.5	0.3 + 0.03	228.2
2–2.5	7.0 ± 0.4	0.2 ± 0.02	398.9
2.5–3	6.4 + 0.3	0.2 + 0.01	458.3
>3	5.5 + 0.3	0.1 + 0.01	140.3
All elevation bands	5.4 ± 0.2	0.2 ± 0.01	1380.7

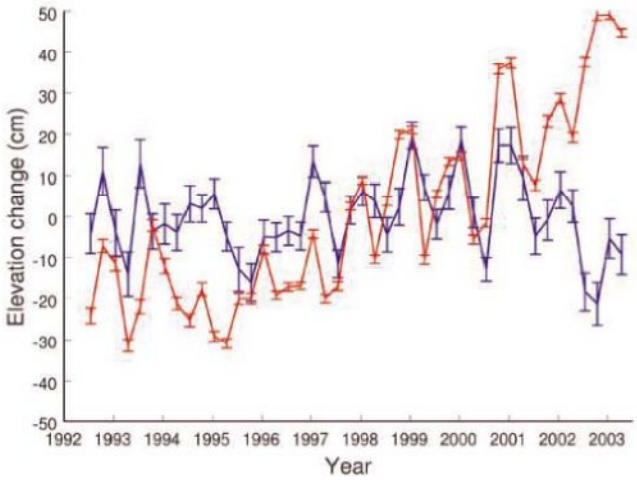

Fig. 2. Interannual variability of spatially averaged Greenland Ice Sheet elevation, shown as anomalies from the 11-year mean, 1992 to 2003. The data are aggregated into areas >1500 m elevation (red) and <1500 m (blue), indicating divergent trends since 2000. The vertical bars indicate SE when averaging the results for each cell.

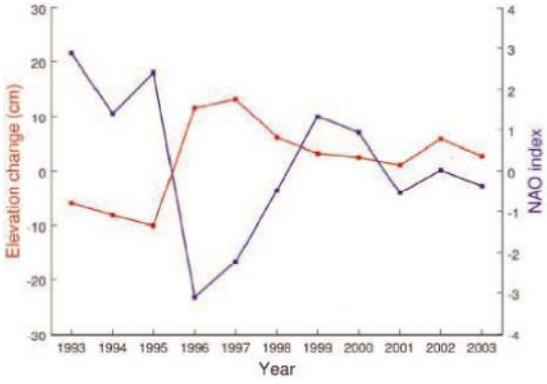

Fig. 3. Spatially averaged changes in winter Greenland Ice Sheet elevation (red) and winter NAO index (blue), lagged 1 month, 1992 to 2003. Winter elevation change during, e.g., 1994/1995 was determined by subtracting autumn 1994 from winter 1994/1995. For elevation, winter is defined as December-January-February with, e.g., winter 1994/1995 specified as 1995. The correlation coefficient between elevation change and the NAO index is –0.88 when lagged 1 month, e.g., November-December-January for the NAO and December-January-February for elevation.

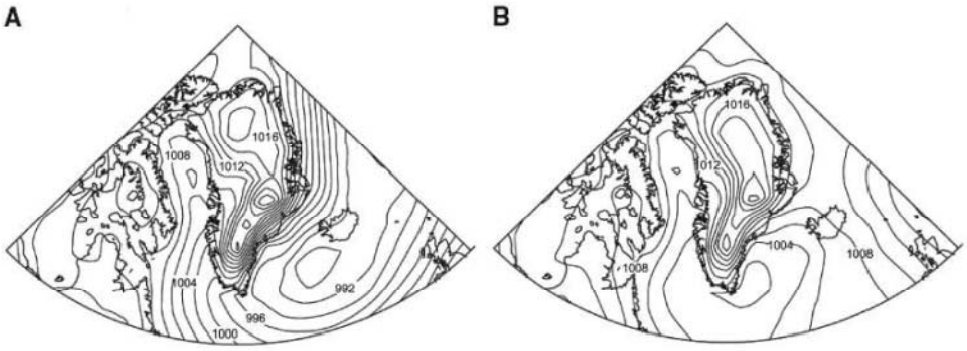

Fig. 4. Composite winter sea-level pressure (mb) in Greenland and surrounding areas (A) 1994/1995 and (B) 1995/1996, which have positive and negative NAO index values, corresponding to negative and positive changes in Greenland Ice Sheet surface elevation, respectively (see Fig. 3). Data are from National Centers for Environmental Prediction (NCEP)/National Center for Atmospheric Research (NCAR) Reanalysis (*35*).

strongly affects ice-sheet elevation change. However, systematic precipitation measurements are available almost exclusively for the coastal stations and not the interior, such that the NAO index may serve as a proxy for precipitation. Therefore, we examine the direct relation between Greenland Ice Sheet elevation change and the NAO index (*16*). Elevation changes during winter have been calculated from the time series using the differences between winter (December-January-February) and the preceding autumn (September-October-November). Figure 3 shows ice-sheet elevation changes during winter and the winter NAO index for 1992 to 2003. The correlation between elevation changes and the NAO is maximum when lagged one month, e.g., November-December-January for the NAO and December-January-February for elevation, with $r \sim -0.88$ ($s < 0.05$, df = 10), thus explaining about three-quarters ($r^2 \sim 0.77$) of the elevation changes. The correlations for spring, summer, and autumn are, as expected, lower: 0.04, –0.08, and –0.28, respectively, implying no significant effect of the NAO during these seasons. The winter correlation (–0.88) is stronger than the above-mentioned correlations for the NAO and modeled Greenland precipitation (*28*), which implies that the NAO index is a very good proxy for winter precipitation data. Therefore, strongly negative NAO-index conditions lead to increased accumulation and elevation change during wintertime, and vice versa. This is exemplified by the changes observed from 1994/1995 (−10.1 cm) to 1995/1996 (+11.6 cm), associated with a record positive-to-negative NAO reversal (2.4σ to −3.1σ) (Fig. 3).

The relation is based not only on the intensity of the NAO but also on the development and position of the Icelandic Low (*29*), which, for example, shifted southwestward to Cape Farewell between 1994/1995 and 1995/1996 (Fig. 4), giving higher precipitation especially in southern Greenland. However, in other years, a weak negative NAO index may be due simply to a weakly developed Icelandic Low, in which case the elevation change is barely positive, as in 2001 (Fig. 3). The relationship appears weak in the most recent years, since 2001, with the NAO index relatively neutral.

The observed correlation between the NAO and ice-sheet elevation changes suggests that future trends in the NAO could influence the Greenland Ice Sheet surface elevation. The winter NAO index trend has been generally positive since the 1960s, although during our 1992 to 2003 study period, the trend happened to be slightly negative, hence the observed increase in elevation. Model experiments with increasing atmospheric concentrations of GHGs generally indicate an increasing (positive) NAO and a slight northeastward displacement of the Icelandic Low in the future (*17*, *18*)—both implying less winter accumulation over Greenland.

Nonetheless, as mentioned, the NAO can explain about three-quarters of the surface elevation changes, leaving us to speculate on other factors. A modeling study (*30*) of the Greenland Ice Sheet mass balance under greenhouse global warming has shown that temperature increases up to 2.7°C lead to positive mass-balance changes at high elevations (due to accumulation) and negative at low elevations (due to runoff exceeding accumulation), consistent with our findings, which implies that perhaps a quarter of the growth may be caused by global warming in Greenland (*31*) in our observation period. Furthermore, the observed elevation change implies that ice-sheet growth in the interior of Greenland may partly offset the freshwater flow of the retreating subpolar glaciers needed to explain the freshening rate of the world ocean, which can be explained almost entirely by Arctic sea-ice melt (*32*).

In conclusion, we have presented new evidence of (i) decadal increase in surface elevation (~5 cm/year) within a study area comprising most of the Greenland Ice Sheet, 1992 to 2003, caused by accumulation over extensive areas in the interior of Greenland; (ii) divergence in elevation changes since the year 2000 for areas above and below 1500 m, with high-elevation increases and low-elevation decreases, the former in contrast to previous research (*10*, *13*); and (iii) negative correlation between winter elevation changes and the NAO index, suggesting an underappreciated role of the winter season and the NAO for elevation changes—a wild card in Greenland Ice Sheet mass-balance scenarios under global warming.

There are, however, caveats to consider. First, we cannot make an integrated assessment of elevation changes—let alone ice volume and its equivalent sea-level change—for the whole Greenland Ice Sheet, including its outlet glaciers, from these observations alone, because the marginal areas are not measured completely using ERS-1/ERS-2 altimetry (see Fig. 1). It is conceivable that pronounced ablation (e.g., *10*, *11*) in low-elevation marginal areas could offset the elevation increases that we observed in the interior areas. Second, there is large interannual to decadal variability in the high-latitude climate system including the NAO, such that the 11-year-long data set developed here remains too brief to establish long-term trends. Therefore, there is clearly a need for continued monitoring using new satellite altimeters—including advanced ones with improved ice-sheet ranging in steeper coastal areas—and other remote-sensing and field observations, together with numerical modeling to calculate the mass budget through net losses and net input from snow (*33*).

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Ancient DNA from the First European Farmers in 7500-Year-Old Neolithic Sites

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The ancestry of modern Europeans is a subject of debate among geneticists, archaeologists, and anthropologists. A crucial question is the extent to which Europeans are descended from the first European farmers in the Neolithic Age 7500 years ago or from Paleolithic hunter-gatherers who were present in Europe since 40,000 years ago. Here we present an analysis of ancient DNA from early European farmers. We successfully extracted and sequenced intact stretches of maternally inherited mitochondrial DNA (mtDNA) from 24 out of 57 Neolithic skeletons from various locations in Germany, Austria, and Hungary. We found that 25% of the Neolithic farmers had one characteristic mtDNA type and that this type formerly was widespread among Neolithic farmers in Central Europe. Europeans today have a 150-times lower frequency (0.2%) of this mtDNA type, revealing that these first Neolithic farmers did not have a strong genetic influence on modern European female lineages. Our finding lends weight to a proposed Paleolithic ancestry for modern Europeans.

Agriculture originated in the Fertile Crescent of the Near East about 12,000 years ago, from where it spread via Anatolia all over Europe (*1*). It has been widely suggested that the global expansion of farming included not only the dispersal of cultures but also of genes and languages (*2*). Archaeological cultures such as the Linear pottery culture (*Linearbandkeramik* or LBK) and Alföldi Vonaldiszes Kerámia (AVK) mark the onset of farming in temperate regions of Europe 7500 years ago (*3*). These early farming cultures originated in Hungary and Slovakia, and the LBK then spread rapidly as far as the Paris Basin and the Ukraine (*4*, *5*). The remarkable speed of the LBK expansion within a period of about 500 years, and the general uniformity of this archaeological unit across a territory of nearly a million square kilometers (Fig. 1), might indicate that the spread was fueled to a considerable degree by a migration of people (*6*–*8*). On the other hand, a number of archaeological studies suggest that local European hunter-gatherers had shifted to farming without a large-scale uptake of genes from the first farmers (*9*–*11*). Genetic studies carried out on modern Europeans have led to conflicting results, with estimates of Neolithic input into the present population ranging from 20 to 100% (*12*–*20*). A theoretical simulation study by Currat and Excoffier (*21*) has recently suggested a minor contribution, clearly less than 50%, and possibly much less. Conclusive ancient DNA studies on skeletons of the first European farmers have so far not been published to our knowledge.

To resolve the question regarding the extent of the Neolithic female contribution to the present European population, we collected 57 Neolithic skeletons from 16 sites of the LBK/AVK culture from Germany, Austria, and Hungary. These include well-known archaeological sites such as Flomborn, Schwetzingen, Bilsleben, Aspern-Schletz, and several new excavations; for example, from Halberstadt and Derenburg Meerenstieg II. All human remains were dated to the LBK or AVK period (7500 to 7000 years ago) on the basis of associated cultural finds. We extracted DNA from bone and teeth from the morphologically well-preserved individuals, and we amplified nucleotide positions (nps) 15997 16409 [see supporting online material (*22*)] of the mitochondrial genome with four overlapping primer pairs. In addition, we typed a number of coding-region mtDNA polymorphisms, which are diagnostic for major branches in the mtDNA tree (*22*).

From a total of 57 LBK/AVK individuals analyzed, 24 individuals (42%) revealed reproducibly successful amplifications of all four primer pairs from at least two independent extractions usually sampled from different parts of the skeleton. Eighteen of the sequences belonged to typical western Eurasian mtDNA branches; there were seven H or V sequences, five T sequences, four K sequences, one J sequence, and one U3 sequence (table S1). These 18 sequences are common and widespread in modern Europeans, Near Easterners, and Cen-

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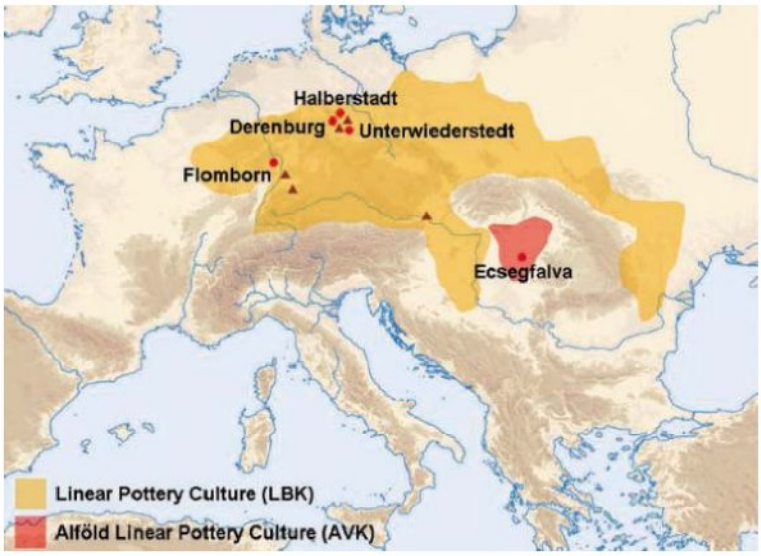

Fig. 1. Geographic range of the first Central European farmers. The orange and red areas indicate the widest distribution of the earliest Neolithic farming cultures LBK and AVK after 7500 years before the present. Circles represent sites with N1a haplotypes, and triangles represent sites with other haplotypes. Names are given for N1a sites only. For details on the archaeological sites, see table S3.

Table 1. mtDNA sequences of the six Neolithic N1a types. Sequences are presented as variant nucleotide positions relative to the Cambridge Reference Sequence (*31*). Nucleotide positions are given, less 16000.

Individual	ID no.	mtDNA sequence 15997–16409
Derenburg 1	DEB1	147.A 172.C 223.T 248.T 355.T
Derenburg 3	DEB3	147.A 172.C 223.T 248.T 320.T 355.T
Halberstadt 2	HAL2	086.C 147.A 172.C 223.T 248.T 320.T 355.T
Flomborn 1	FLO1	147.A 172.C 223.T 248.T 320.T 355.T
Unterwiederstedt 5	UWS5	129.A 147.A 154.C 172.C 223.T 248.T 320.T 355.T
Ecsegfalva 1	ECS1	147.A 172.C 189.C 223.T 248.T 274.A 355.T

tral Asians, and thus these 18 lineages lack the detailed temporal or geographic discrimination required to test the hypotheses we are examining, even though some of them have previously been suggested to be of Neolithic origin on the basis of modern DNA studies (*15*). We therefore concentrated on the mtDNA types identified in the other six individuals.

The most striking result is that 6 of the 24 Neolithic skeletons are of the distinctive and rare N1a branch. For verification, we sequenced 517 clones derived from independent extractions from different parts of the six individuals. All six showed the suite of mutations characteristic of the N1a lineage. Five of these six individuals display different N1a types, whereas Flomborn 1 and Derenburg 3 show identical N1a types (Table 1).

The observed distinct N1a types rule out the possibility of contamination with modern samples, which can be a problem in ancient human DNA studies. It is implausible that the five types are from five different modern contaminants, because the frequency of this type today is very low anywhere in the world, at about 0.2% (*23*, *25*) (fig. S1). It is also unlikely that the sequence variations seen within the five N1a types are the result of random postmortem DNA damage (*26*, *27*), because three out of six sequence types that we have identified precisely match modern sequences previously published in the literature (table S2 and supporting references); finally, two further N1a types (HAL2 and UWS5) precisely fit into predicted but previously unobserved ancestral nodes in the N1a phylogeny (Fig. 2), underlining the authenticity of the ancient DNA.

The high frequency of our Neolithic N1a lineages is not a local phenomenon but is widespread in the LBK area: Independently sampled locations in Hungary and Germany, over 800 km apart, each yielded one or more N1a types (Fig. 1). The modern geographic spread of N1a types partly reflects the Neolithic situation, albeit at a much lower modern frequency: All Neolithic LBK types fall into the "European" N1a sub-branch, and this sub-branch today is rare but widespread in Europe and adjacent parts of Asia and North Africa (Fig. 3). The AVK sample ECS1 shows 16189C, which is characteristic of the Central Asian branch, but in this case is plausibly a parallel mutation in the European branch, because position 16189 mutates much more rapidly than the conflicting 16320 position (*28*).

We next addressed the question of whether the 150-times lower frequency of N1a in modern Europeans might be due to simple genetic drift over the past 7500 years. Given a frequency of N1a within our Neolithic sample of 25%, the frequency in the Neolithic LBK population is estimated to lie between 8% and 42% (95% confidence interval, based on binomial standard error). Even the lower limit of 8% contrasts markedly with an N1a frequency of 0.2% (5 in 2300) in modern mtDNA samples in the LBK area between the Paris Basin and Hungary. Qualitatively, modern Europeans therefore do not appear to be maternally descended from the first farmers. However, there remains a possibility that modern European maternal lineages are descended from the early farmers but that the N1a type has been lost during the past 7500 years through genetic drift. We therefore applied computer simulations to test whether the frequency of the Neolithic N1a types could have been drastically decreased by drift alone in the past 7500 years.

We simulated a scenario that would maximize the chance that N1a has been lost by genetic drift in the course of the past 7500 years. The simulation showed that we should observe at least 74 N1a's out of the 2300 modern samples. In fact, 95% of the total runs ended showing between 119 and 259 N1a's in the modern sample. Next, we allowed migration between the Neolithic population and the surrounding population per generation. The simulation showed that a migration rate of 1% per generation throughout 7425 years between the Neolithic population and the surrounding population is not enough to reduce the N1a percentage to the low value observed today, because only 5.5% of the total runs ended in <6 N1a's in the modern sample.

These simulations reject the simple hypothesis in which modern Europeans are direct descendants of these first farmers and have lost N1a mainly by genetic drift. Hence the simulations confirm that the first farmers in Central Europe had limited success in leaving a genetic mark on the female lineages of modern Europeans. This is in contrast to the success of the Neolithic farming culture itself, which subsequently spread all over Europe, as the archaeological record demonstrates. One possible explanation is that the farming culture itself spread without the people originally carrying these ideas. This includes the possibility that small pioneer groups carried farming into new areas of Europe, and that once the technique had taken root, the surrounding hunter-gatherers adopted the new culture and then outnumbered the original farmers, diluting their N1a frequency to the low modern value. Archaeological research along the Western periphery of LBK and isotope studies of some of our sampled individuals seem to support the idea that male and female hunter-gatherers were integrated into the Neolithic communities (*3*, *10*, *29*). This hypothesis implies that N1a was rare or absent in Mesolithic Europeans, which may be a reasonable assumption given the rarity of the N1a type anywhere in the world (Fig. 3). An alternative hypothesis is a subsequent post early-Neolithic population replacement in Europe,

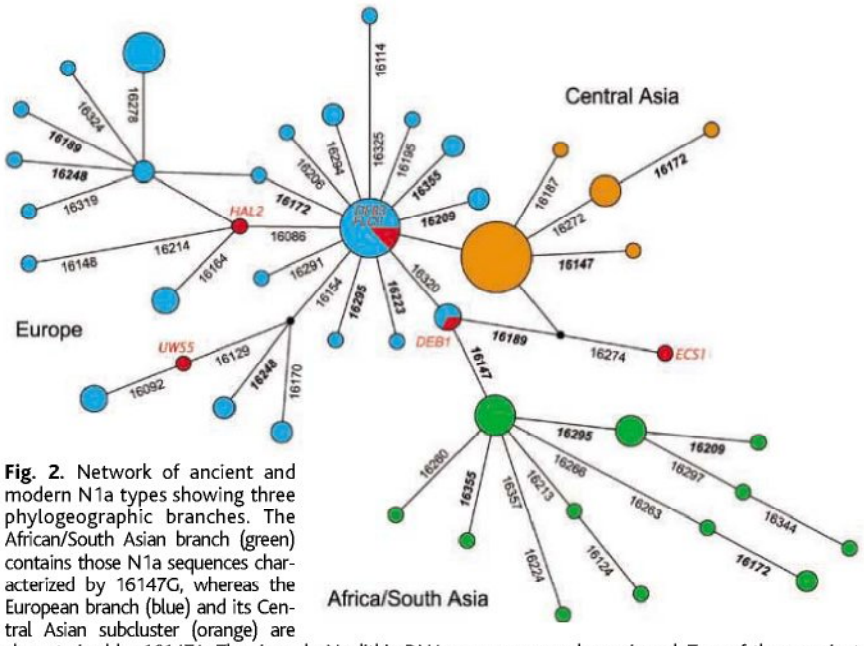

Fig. 2. Network of ancient and modern N1a types showing three phylogeographic branches. The African/South Asian branch (green) contains those N1a sequences characterized by 16147G, whereas the European branch (blue) and its Central Asian subcluster (orange) are characterized by 16147A. The six early Neolithic DNA sequences are shown in red. Two of these ancient farmers (HAL2 and UWS5) fall into hitherto unsampled but predicted nodes, further confirming the authenticity of the ancient DNA. The Central Asian subcluster is at least 2500 years old, because the nodal Central Asian N1a type had been found in a Scytho-Siberian burial in the Altai region (*30*). Circles and pie-slice sizes are proportional to frequencies, and mutated nucleotide positions are shown along the branches.

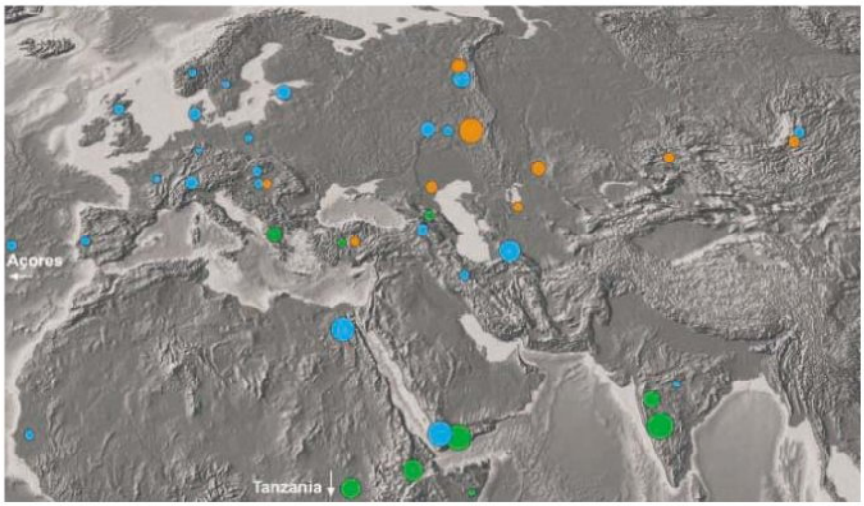

Fig. 3. Modern geographic spread of the three N1a branches. Blue circles depict the European branch of N1a, orange circles the Central Asian branch of N1a, and green circles the African/South Asian branch. The three N1a branches are defined in the network of Fig. 2. The smallest circle size corresponds to a local frequency of 0.18%, and larger frequencies are indicated by proportionately larger circles.

eliminating most of the N1a types. Archaeological evidence for such an event is as yet scant.

The results from the Neolithic sample show that other mtDNA lineages considerably diluted the mtDNA pool of these early Neolithic populations, so that the frequency of N1a in modern Europeans is 150 times lower than in our sample of the first Central European farmers. This is incompatible with the idea that modern Central Europeans—and by implication other Europeans beyond the LBK/AVK area—derive their maternal lineages purely from the earliest farmers of that region. Within the current debate on whether Europeans are genetically of Palaeolithic or Neolithic origin, and leaving aside the possibility of significant post-Neolithic migration, our data lend weight to the arguments for a Palaeolithic origin of Europeans.

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Photosynthetic O_2 Formation Tracked by Time-Resolved X-ray Experiments

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Plants and cyanobacteria produce atmospheric dioxygen from water, powered by sunlight and catalyzed by a manganese complex in photosystem II. A classic S-cycle model for oxygen evolution involves five states, but only four have been identified. The missing S_4 state is particularly important because it is directly involved in dioxygen formation. Now progress comes from an x-ray technique that can monitor redox and structural changes in metal centers in real time with 10-microsecond resolution. We show that in the O_2-formation step, an intermediate is formed—the enigmatic S_4 state. Its creation is identified with a deprotonation process rather than the expected electron-transfer mechanism. Subsequent electron transfer would give an additional S_4' state, thus extending the fundamental S-state cycle of dioxygen formation.

In plants, algae, and cyanobacteria (blue-green algae), both electrons and protons are extracted from water molecules in a light-driven process denoted as photosynthetic water oxidation (*1*, *2*). Atmospheric dioxygen (O_2) is formed as a by-product. The reactions leading to O_2 formation proceed at a tetramanganese complex bound to the proteins of photosystem II (PSII) and involve a nearby tyrosine radical ($Y_Z^{\bullet}$). There has been exciting progress toward elucidating the structure of PSII (*3*–*6*), including a first crystallographic model of the manganese complex (*5*), but the mechanism of O_2 formation has remained obscure.

Since 1970, the paradigm for understanding O_2 evolution has been the S-state cycle proposed by Bessel Kok (*7*). This model involves five oxidation states (S states) of the PSII donor side. Four of these are stable for several seconds (S_0, S_1, S_2, and S_3) and have been characterized, but evidence for an S_4 intermediate is lacking. In the S-cycle model, O_2 formation requires successive absorption of four light quanta that can be provided by short flashes of light. By driving electron transfer from donor to acceptor side of PSII, each flash initiates an S-state transition until the S_4 state is reached (Fig. 1). Subsequently, dioxygen is released. The S_0 state is concomitantly formed, as the previously accumulated oxidizing equivalents are used for O-O bond formation. The elusive S_4 state is a transiently formed intermediate of O_2 formation in the $S_3 \Rightarrow S_0$ transition. It is particularly important because it represents the starting point for O-O bond formation. Because the S_1 state is dark-stable (ground state), the first four laser flashes drive the transitions $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \Rightarrow S_0$, and $S_0 \rightarrow S_1$. Dioxygen is formed on the third flash in the $S_3 \Rightarrow S_0$ transition, but with the lack of identification of an S_4 intermediate, this crucial transition is poorly understood.

Recently, the O_2-formation step in PSII from cyanobacteria was inhibited by high oxygen pressure and, on the basis of ultraviolet absorption (UV) changes, it was proposed that an intermediate state had been stabilized (*8*). Pitfalls of this elegant experiment are the uncertain role of the proposed intermediate at ambient oxygen pressure and the unclear relation between UV absorption changes and the redox state of the PSII manganese complex. Instead of attempting to trap intermediates by inhibiting dioxygen formation, we took the course of monitoring the redox processes at the fully functional manganese complex in real time by time-resolved x-ray absorption spectroscopy (XAS). We investigated highly active PSII in its native membrane environment at ambient oxygen pressure and temperature and were able to identify the enigmatic S_4 state formed before O-O bond formation. In addition, we propose an extension of the classic S-state cycle (Fig. 1).

XAS at the K edge of manganese probes changes in oxidation state and local structure specifically for the x-ray absorbing Mn ions (*9*, *10*). Thus, time-resolved XAS is ideal for monitoring the kinetics of the individual steps in the catalytic cycle of the PSII manganese complex. XAS experiments involving a freeze-quench technique have been used to study alcohol dehydrogenase intermediates with a time resolution of 3 ms (*11*, *12*). Tracking the faster S-state

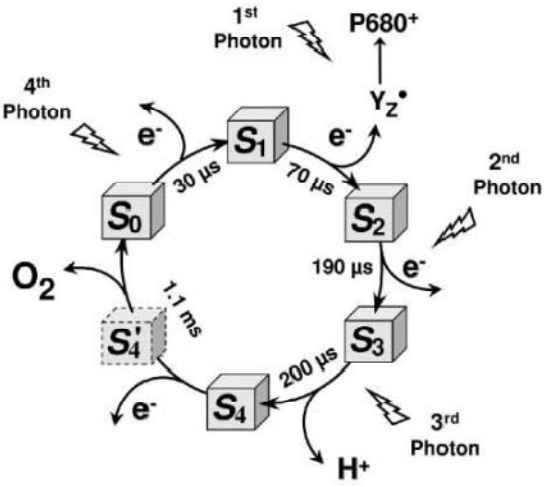

Fig. 1. Extension of the classic S-state cycle of photosynthetic oxygen evolution. The classic S-cycle model has been proposed by Kok (*7*) on the basis of the flash-number dependence of the O_2 yield that was first observed by Joliot and Joliot (*26*). The oxygen-evolving complex (OEC) at the PSII donor side comprises a manganese-calcium complex (4 Mn and 1 Ca) and its protein environment. Often also a nearby tyrosine (Y_Z) is included as an integral part of the OEC (*1*–*6*). Driven by the sequential absorption of four light quanta, which in the present study were provided by four laser flashes, the OEC is stepped through its reaction cycle. After absorption of a photon, a chlorophyll cation (P_{680}^{+}) is formed, which oxidizes Y_Z. The tyrosine radical ($Y_Z^{\bullet}$) then extracts one electron from the Mn complex. The S_1 state is dark-stable; S_2 and S_3 are formed by one and two light-driven oxidation steps, respectively. The third photon induces the $S_3 \rightarrow S_0$ transition and dioxygen is released; the fourth photon closes the cycle. Proton release (*27*) not representing a distinct, rate-limiting step has been omitted. Existence and formation rate of the S_4 state are uncovered in the present investigation. The S_4 intermediate is not formed by electron transfer to $Y_Z^{\bullet}$ but by a deprotonation reaction. In S_4, four oxidizing equivalents have been accumulated by the OEC, including $Y_Z^{\bullet}$. The classic S-state cycle is extended by the S_4' state that represents a hypothetical intermediate in which four electrons have been extracted from the Mn complex, including Mn ligands and the two substrate water molecules.

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transitions of the PSII manganese complex required an improvement in time resolution by more than two orders of magnitude. On the basis of our studies of the semistable S states by XAS at room temperature [(*13*, *16*); for the first flash-and-freeze study, see (*17*)], we have developed an approach (*16*) that makes possible the direct observation of S-state transitions by XAS.

Facilitated by the high flux and stability of the x-ray beam at a third-generation synchrotron (European Synchrotron Radiation Facility, beamline ID26, Grenoble, France), we could follow changes in the Mn x-ray fluorescence after laser-flash illumination of PSII with a time resolution of 10 μs (Fig. 2). At an excitation energy of 6552 eV, for $S_1 \rightarrow S_2$ (first flash) and $S_2 \rightarrow S_3$ (second flash) the exponential absorption decrease indicated oxidation of the Mn complex by the tyrosine radical $Y_Z^{\bullet}$ with halftimes of 70 μs and 190 μs, respectively; for $S_0 \rightarrow S_1$ (fourth flash), the $t_{1/2}$ was ≤30 μs.

For $S_3 \Rightarrow S_0$ (third flash), the laser flash induced an absorption increase due to Mn reduction by the substrate water ($t_{1/2}$ = 1.1 ms); however, this was preceded by a lag phase of about 250 μs (Fig. 2). This lag phase suggested a kinetically resolvable intermediate. However, for transients collected at 6552 eV, it could not be unambiguously assigned to an intermediate in the $S_3 \Rightarrow S_0$ transition. An absorption-change contribution from a minor fraction of PSII that undergoes the $S_2 \rightarrow S_3$ transition on the third flash (due to PSII that did not turn over on the first laser flash, the so-called "misses") might mimic a lag phase. A time-resolved XAS experiment at 6556 eV clarified the situation because at this energy, no change in the x-ray absorption is observed for $S_2 \rightarrow S_3$ (*15*). A sizable lag phase was still present (Fig. 3), proving the existence of a kinetically resolvable intermediate in the transition from the $S_3Y_Z^{\bullet}$ to the S_0Y_Z state. The intermediate is formed before the Mn-reducing/O_2-forming step and thus represents the long-searched-for S_4 state (SOM Text). Two advantages of the XAS approach facilitated the discovery of S_4. First, in contrast to previous studies that used visible light instead of x-rays to probe reactions in PSII, absolute manganese specificity is ensured. Second, the trace collected at an isosbestic point of the $S_2 \rightarrow S_3$ transition (6556 eV) removes the ambiguities due to imperfect turnover on the previous S-state transition. Of note is that the predicted peroxidic S_2* intermediate (*8*) was not observed at ambient oxygen pressure. The reaction sequence $S_1 \rightarrow S_2$*$\rightarrow S_0$ could lead to a biphasic absorption increase on the third flash in the time-resolved experiment at 6552 eV, but Figs. 2 and 3 indicate monophasic Mn reduction.

Three observations enable us to identify the chemical nature of the S_4 intermediate: (i) Formation of a Mn-oxo species (Mn^V=O,

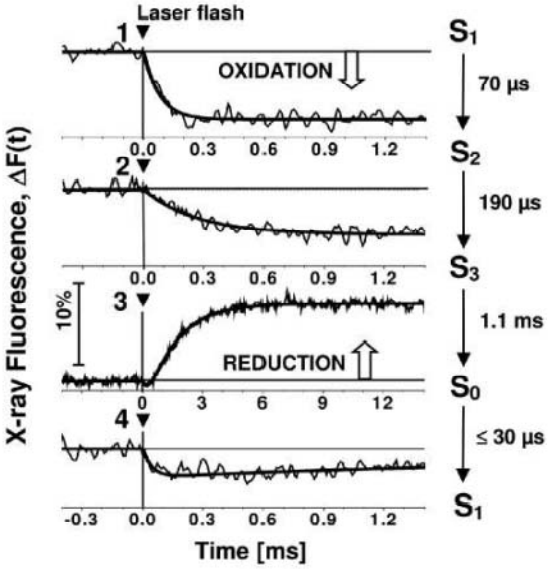

Fig. 2. Oxidation and reduction of the Mn complex of PSII monitored by time-resolved x-ray measurements. Nanosecond flashes of green laser light initiated the S-state transitions. For 5 ms before and 15 ms after each flash, the protein samples were exposed to x-rays of 6552 eV. The time course of the Mn K_α fluorescence was recorded, because the intensity of the x-ray fluorescence measures the probability for x-ray absorption at the chosen excitation energy [normalization of $\Delta F(t)$ to an edge-jump of unity as described in (*15*)]. The time resolution was 10 μs per data point; five data points were averaged for the third-flash data. At 6552 eV, oxidation/reduction of the PSII manganese complex results in decrease/increase of $F(t)$ (fig. S1). Monoexponential (first and second flashes) or biexponential (third and fourth flashes) simulations led to the solid lines and the indicated halftimes that correspond to the following first-order rate constants (in s^{-1}): 9.9×10^3 ($S_1 \rightarrow S_2$), 3.6×10^3 ($S_2 \rightarrow S_3$), 6.3×10^2 (reduction on $S_3 \rightarrow S_0$), and $\geq 2 \times 10^4$ ($S_0 \rightarrow S_1$).

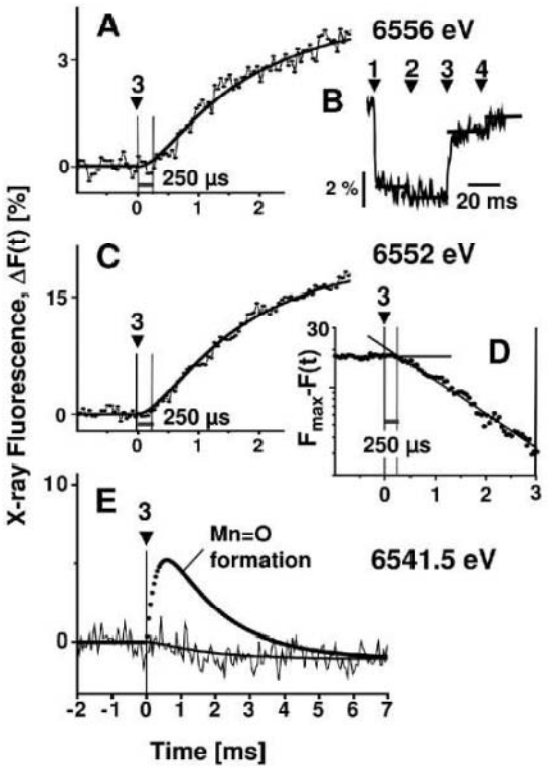

Fig. 3. Intermediate formation in the oxygen-evolving $S_3 \rightarrow S_0$ transition induced by the third laser flash. In (**A**), x-ray fluorescence changes, ΔF, are shown for x-ray excitation at 6556 eV. At this energy, ΔF is zero for the $S_2 \rightarrow S_3$ transition (*15*), as demonstrated in (**B**). (The minor second-flash change visible in (**B**) is due to the 14% of PSII that "missed" the first flash and therefore undergoes the $S_1 \rightarrow S_2$ transition on the second flash.) Thus, at an excitation energy of 6556 eV, the lag phase of ~250 μs duration can be unambiguously assigned to intermediate formation; correction for miss events on preceding flashes is not required. In (**C**), the x-ray fluorescence transient measured at 6552 eV was corrected for miss contributions leading to the same lag phase behavior as observed at 6556 eV. For the corrected transient shown in (**C**), the logarithmic plot in (**D**) demonstrates the duration of the lag phase and the monophasic Mn reduction thereafter. A logarithmic plot for the transient in (**A**) yielded the same picture (fig. S4). In (**E**), a transient measured at 6541.5 eV (pre-edge feature) is compared to the time course expected for Mn–O formation (fig. S5). The smooth lines in (**A**), (**C**), and (**E**) were obtained by simulations for a consecutive reaction scheme with $k_1 = 3.3\ ms^{-1}$ and $k_2 = 0.62\ ms^{-1}$.

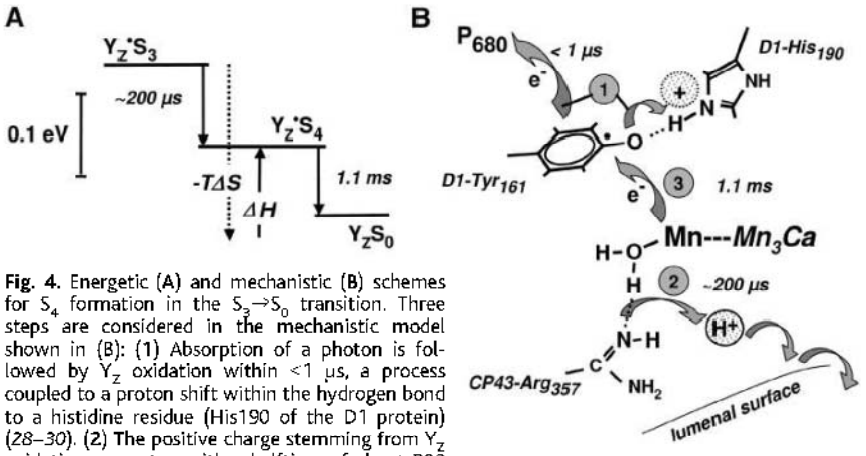

Fig. 4. Energetic (**A**) and mechanistic (**B**) schemes for S_4 formation in the $S_3 \rightarrow S_0$ transition. Three steps are considered in the mechanistic model shown in (B): (**1**) Absorption of a photon is followed by Y_Z oxidation within <1 μs, a process coupled to a proton shift within the hydrogen bond to a histidine residue (His190 of the D1 protein) (*28*–*30*). (**2**) The positive charge stemming from Y_Z oxidation promotes, with a halftime of about 200 μs, proton shifts and deprotonation of Arg357 of the CP43 protein. This assignment of the deprotonating group is tentative, but plausible (*23*). The proton is moving in a bucket-brigade-type mechanism along the proton path identified in (*5*) toward the lumenal surface. S_4 formation by deprotonation of the Mn complex may enable the subsequent reaction steps in two ways. (i) The redox potential of the Mn complex is lowered, facilitating electron transfer to $Y_Z^{\bullet}$. (ii) The deprotonated group acts as a proton acceptor in the O-O bond formation step (*25*). (**3**) S_4 formation is followed by electron transfer to $Y_Z^{\bullet}$. This process formally corresponds to formation of the S_4' state in Fig. 1 but is kinetically indistinguishable from Mn reduction and O_2 formation (identical halftimes of ~1.1 ms).

oxygen connected by a double bond to five-fold oxidized manganese) before the water-oxidation step has been proposed [e. g., in (*18*)], but this possibility can be excluded because it would give a transient rise of the pre-edge amplitude at 6541.5 eV, which is not observed (Fig. 2E). (ii) The same lag-phase behavior is observed throughout the edge region (fig. S6), indicating S_4 formation without Mn oxidation or structural changes of the Mn complex. The lag phase is also shorter than the millisecond halftime of electron transfer to $Y_Z^{\bullet}$ (*19*, *20*), which matches the $t_{1/2}$ of Mn reduction. Thus, the oxidation states of the Mn complex and of $Y_Z^{\bullet}$ remain unchanged upon S_4 formation. (iii) We measured recombination fluorescence emitted by the chlorophyll antenna of PSII and show that the Gibbs free energy of S_4 formation is pH-dependent and exhibits an H_2O/D_2O exchange effect (figs. S7 and S8). The temperature dependence indicates that S_4 formation is entropically driven, as predicted for proton release into the bulk-phase water (ΔG = –0.1 eV or –10 kJ/mol at pH 6.4 and 25°C; ΔH = –0.1 eV; $T\Delta S$ = –0.2 eV). An S_4 formation by deprotonation and proton release into the bulk-phase water can explain the recombination-fluorescence results as well as the x-ray absorption lag. The x-ray absorption is increasing with the onset of Mn reduction after deprotonation of a base close to the Mn complex. This conjecture is consistent with proton-release measurements (*21*) and electrochromism studies (*22*).

On the basis of the above findings, we do not identify S_4-state formation with electron transfer from the Mn complex to $Y_Z^{\bullet}$, as has been proposed, but with the formation of a base *B* by a deprotonation process. Crystallographic data facilitates a tentative attribution of *B* to Arg357 of the CP43 protein (*23*) (Fig. 4): direct deprotonation of substrate water also is conceivable.

Classical electron transfer (*24*) is not directly coupled to protonation state changes of donor (reductant) or acceptor (oxidant). Hydrogen-atom transfer is the joint movement of proton and electron to an H-atom acceptor (or abstractor). Formation of a Mn^{V}-oxo species (Mn^{V}=O), in the S_4 state, by H-atom transfer from a Mn^{IV}-hydroxo (Mn^{IV}-OH) to the tyrosine radical ($Y_Z^{\bullet} \rightarrow Y_Z$H) has been a centerpiece of an influential hypothesis on photosynthetic water oxidation (*18*). Here, we report evidence for an alternative mechanism, a "proton-first" electron transfer, where the oxidation of Y_Z induces likely electrostatically—a deprotonation reaction that is a prerequisite to the subsequent electron transfer to $Y_Z^{\bullet}$ (*25*).

Our identification of S_1 formation as a deprotonation process bears mechanistic implications and will spur further investigations on this key step in photosynthetic water oxidation. It also leads to an extension of the S-state cycle because the deprotonation must be followed by electron transfer to $Y_Z^{\bullet}$, thus implying an S_1' state (Fig. 1). This time-resolved x-ray experiment takes us a step closer to the goal of watching biological function in real time.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S8
Table S1
References

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Small-Molecule Inhibition of TNF-α

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We have identified a small-molecule inhibitor of tumor necrosis factor α (TNF-α) that promotes subunit disassembly of this trimeric cytokine family member. The compound inhibits TNF-α activity in biochemical and cell-based assays with median inhibitory concentrations of 22 and 4.6 micromolar, respectively. Formation of an intermediate complex between the compound and the intact trimer results in a 600-fold accelerated subunit dissociation rate that leads to trimer dissociation. A structure solved by x-ray crystallography reveals that a single compound molecule displaces a subunit of the trimer to form a complex with a dimer of TNF-α subunits.

Direct inhibition of TNF-α by the commercial biological agents etanercept (Enbrel, Amgen Incorporated, Thousand Oaks, CA, Wyeth Pharmaceuticals, Collegeville, PA), infliximab (Remicade, Centocor, Horsham, PA, Schering-Plough, Kenilworth, NJ), and adalimumab (Humira, Abbott Laboratories, Abbott Park, IL) has produced significant advances in rheumatoid arthritis treatment and validated the extracellular inhibition of this proinflammatory cytokine as an effective therapy. However, despite considerable incentives, viable leads for analogous small-molecule inhibitors of TNF-α have not been reported (*1*). Thus far, small-molecule antagonists of TNF-α activity have typically been limited to inhibitors of the processing enzyme TACE (*2*), uncharacterized inhibitors of TNF-α expression (*3*–*7*), uncharacterized inhibitors of TNF-α cell-based assays (*8*, *9*), and other intracellular pathway inhibitors that antagonize nuclear factor κB (NF-κB), activating protein 1 (AP1), or c-Jun N-terminal kinase (JNK)/p38 signal transduction. Although progress has been made in developing small molecules capable of disrupting such protein-protein interactions, this process remains a very difficult challenge (*10*, *11*).

We discovered a compound (Fig. 1A) composed of trifluoromethylphenyl indole and dimethyl chromone moieties linked by a dimethylamine spacer that inhibited TNF-α receptor binding (*12*). Potency measurements showed a median inhibitory concentration (IC_{50}) of 22 μM for inhibiting in vitro TNF receptor 1 (TNFR1) binding to TNF-α (Fig. 1B). Comparable potency was observed for inhibiting TNF-α–mediated stimulation of inhibitor of NF-κB (IκB) degradation in HeLa cells but not for orthogonal interleukin-1β (IL-1β)–mediated stimulation of the same pathway (Fig. 1C).

The x-ray crystal structure was solved for TNF-α compound complex crystals generated from an equimolar mixture of TNF-α and compound in solution (table S1). Prior efforts to produce diffraction-quality co-crystals by soaking compound into native TNF-α crystals had failed and often resulted in the cracking of those crystals. Molecular replacement with the coordinates from a single subunit of the 1TNF.pdb structure (*13*) readily solved the structure after failures with intact trimer coordinates. This phenomenon was explained when it was revealed that the compound had displaced one of the subunits from the TNF-α trimer (Fig. 2A). The resulting TNF-α dimer retained the same basic structural subunit fold as the native trimer, but the angle between the subunits within the dimer was slightly widened (Fig. 2B). Clear x-ray density showed the compound bound within a shallow pocket (Fig. 2C) and contacting residues from each subunit of the TNF-α dimer (table S2). Remarkably, these contact surfaces are found completely buried within the subunit interfaces of the intact TNF-α trimer crystal structure.

The compound in this structure is in a compact conformation, with the trifluoromethylphenyl indole and dimethyl chromone moieties folded back upon one another. The binding surface for the compound on the TNF-α dimer is composed of 16 contact residues, including 6 tyrosine residues (Fig. 2D). Nine are presented from chain A [L57, Y59, S60, Q61, Y119, L120, G121, G122, and Y151 (*14*)]. The remaining seven are a subset of these residues presented from chain B (L57′, Y59′, S60′, Y119′, L120′, G121′, and Y151′). The identity of the residues that occupy these positions in other trimeric cytokine family members is shown in table S2. Tyr^{119} is notable in being located close to the threefold symmetry axis of the TNF-α trimer and in making contact with its counterparts from the other monomeric subunits. Its chi-1 angle ro-

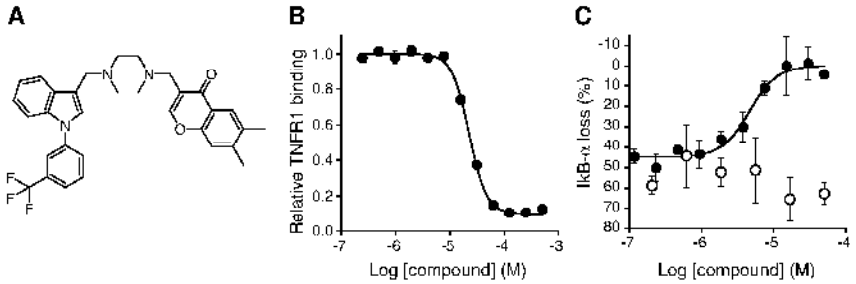

Fig. 1. (**A**) Chemical structure of the small molecule TNF-α inhibitor. See (*12*) for synthetic route used. (**B**) Compound inhibition of TNF-α binding to TNFR1 in vitro. An ELISA (*12*) was used to measure inhibition of solution-phase TNF-R1 horseradish peroxidase conjugate binding to biotinylated TNF-α immobilized on a strepavidin-coated microtiter plate by serial dilutions of compound. The solid line represents a four-parameter curve fit (*20*) that yielded an IC_{50} value of 22 μM. (**C**) Compound inhibition of TNF-α induced IκB-α depletion in HeLa cells. Cells were treated with sufficient TNF-α or IL-1β to give an 80% of maximal IκB-α depletion response after a 30-min exposure (0.4 and 0.04 ng/ml, respectively), as measured in an assay of cell lysates (*12*). Solid circles show that compound addition inhibits this TNF-α–induced IκB-α depletion and yields an IC_{50} value of 4.6 μM. Open circles show that compound addition does not affect orthogonal IL-1β–induced IκB-α depletion. Error bars indicate standard deviations for triplicate measurements.

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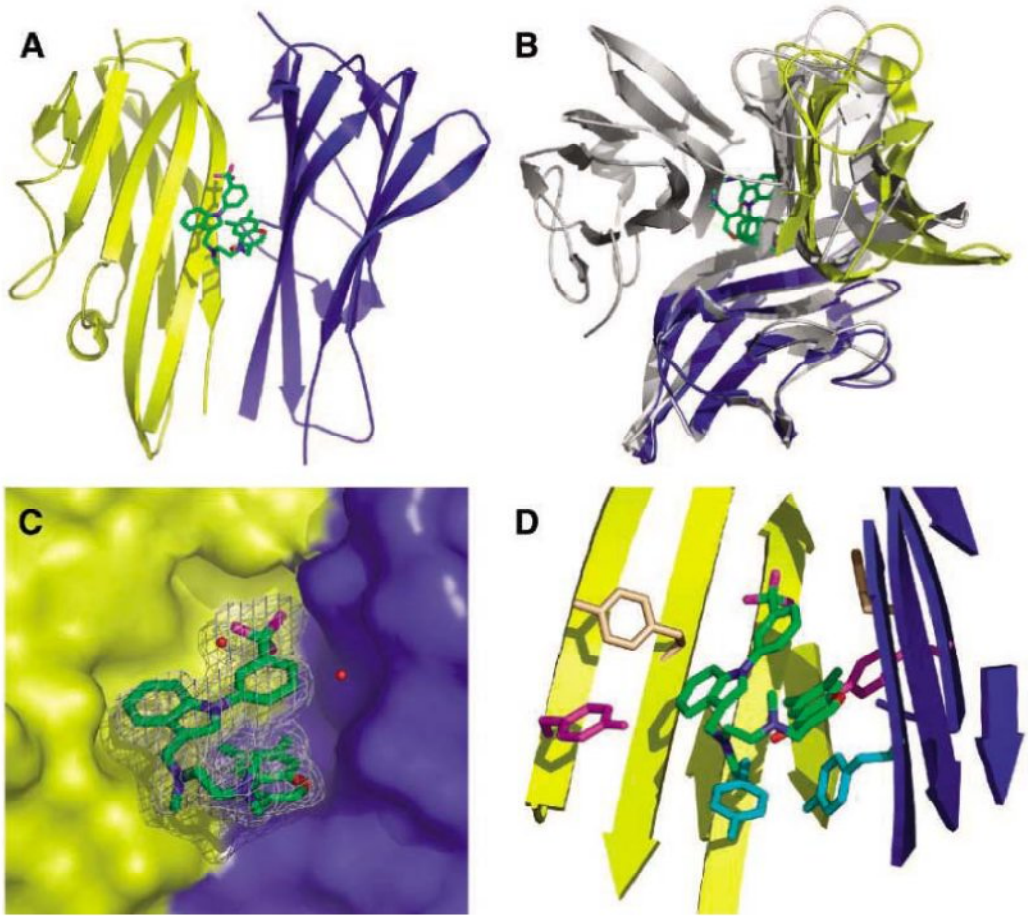

Fig. 2. (**A**) X-ray crystallography structure of the TNF-α dimer–compound complex. (**B**) Shift in subunit orientation within the TNF-α dimer–compound complex. Superposition of the TNF-α trimer structure (gray) with the TNF-α dimer–compound complex structure (yellow-blue) shows a slight widening in the angle between the subunits at the compound binding site. (**C**) View of compound binding site on the TNF-α dimer. Image shows the $2F_o - F_c$ electron density omit map of the compound (contoured to 1σ) calculated from phases derived from refinement of the structure without compound (mesh). The binding pocket can be seen to comprise residues from both chain A (yellow) and chain B (blue). (**D**) β sheet secondary structure around the compound binding site and the location of six tyrosine residues that contact the compound. The tyrosines are colored-coded with Tyr^{59} (tan), Tyr^{151} (purple), and Tyr^{119} (blue) residues being presented from both chain A and chain B.

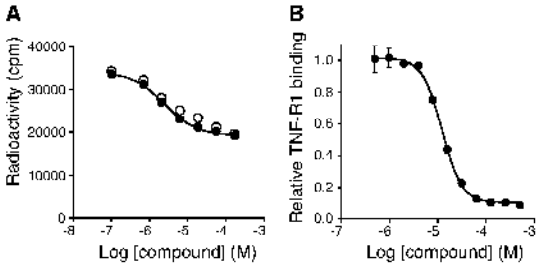

Fig. 3. Displacement of subunits from the TNF-α trimer by compound. (**A**) Singly biotinylated ^{3}H-TNF-α immobilized on a SPA flash plate was exposed to a titration of compound (*12*). Graph shows increasing losses of the immobilized TNF label from the surface (*y* axis) after exposure to increasing compound concentrations for 60 min. Losses measured before washing away dissociated label are shown as open circles, and those after washing are shown as solid circles. (**B**) Singly biotinylated but unlabeled TNF-α immobilized on a strepavidin-coated microtiter plate was likewise exposed to a titration of compound as described (*12*). The microtiter plates were then washed, and functional TNF-α trimer measured by ability to bind TNFR1 peroxidase conjugate. Graph shows increasing losses of the binding competent TNF-α (*y* axis) upon 60 min of exposure to increasing concentrations of compound (IC_{50} ~ 13 μM). Error bars indicate standard deviations for triplicate measurements.

tates 138° and 124° (for chains A and B, respectively) to accommodate compound binding and TNF-α dimer formation. Other side chain movements are relatively minor. In spite of burying about 330 Å^2 (*15*) of protein surface, no intermolecular hydrogen bonds or salt bridges are formed, suggesting that the interaction is largely hydrophobic and shape-driven.

Because the compound contacted residues that are buried in the TNF-α trimer, we examined whether it could dissociate the trimer under the conditions used for the in vitro activity measurements. To this end, we immobilized singly biotinylated ^{3}H-TNF-α trimer onto a scintillation proximity assay (SPA) microtiter plate. Addition of the compound at concentrations that inhibit TNFR1 binding induced shedding of subunits from the TNF-α trimer (Fig. 3A). Experiments measuring the decrease in receptor binding after washing away dissociated subunits gave an IC_{50} of 13 μM (Fig. 3B). This was reproducibly twofold more potent than the value when receptor was present along with the compound (Fig. 1B), consistent with the notion that TNFR1 binding can stabilize TNF-α trimer.

Because the crystal structure indicated that the compound should induce formation of TNF-α dimer, we examined whether this could be observed in solution. To this end, we used mass spectrometry to examine the oligomeric state of TNF-α in the presence of the compound (*12*). Conditions were established that allowed the measurement of the noncovalently associated TNF-α trimer (Fig. 4A). Compound addition under these conditions resulted in the conversion of TNF-α trimer to dimer (Fig. 4B). Additionally, about 20% of the TNF-α dimer observed existed as a complex with a single compound molecule, thereby reproducing the stoichiometry observed in the x-ray structure. Lastly, we performed hydrogen-deuterium exchange experiments to check for the expected increase in exposed surface area per TNF-α subunit in the dimer form. Measurements showed that addition of compound

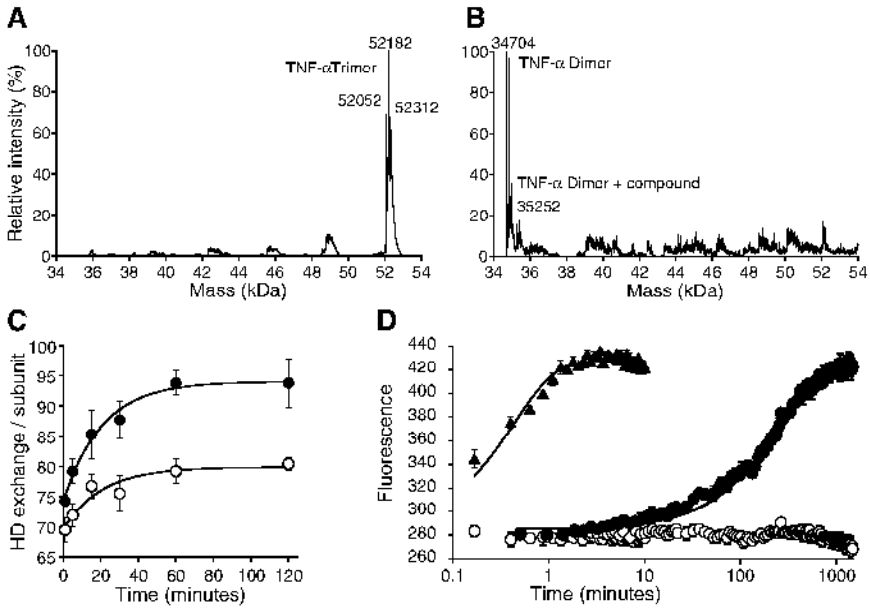

Fig. 4. Data showing compound-induced formation of TNF-α dimer in solution. **(A)** Detection of TNF-α oligomeric state by mass spectrometry. Spectrum is a deconvoluted neutral scale mass spectrum of noncovalent TNF-α trimer complex visualized as three peaks due to plus and minus N-terminal methionine heterogeneity of TNF-α sample. The observed masses of 52,052, 52,182, and 52,312 daltons corresponds to the trimer complex containing either zero, one, or two subunits with N-terminal methionines, respectively. **(B)** Analogous spectrum of 10 μM TNF-α incubated with 100 μM SP307. Masses at 34,704 and 35,252 correspond to noncovalent complex of TNF-α dimer and TNF-α dimer–compound, respectively. **(C)** Increase in TNF-α exchangeable hydrogens induced by compound. Plot shows time course for hydrogen-deuterium exchange of 1 μM TNF-α dissolved into either D_2O alone (open circles) or D_2O plus 30 μM compound (solid circles). Procedure was performed as described (*12*). Calculations of exposed surface area predict 88, 99, and 110 exchangeable hydrogens for each subunit within the TNF-α trimer, dimer, and monomer structures, respectively (*21*, *22*). **(D)** TNF-α subunit dissociation rates in the presence and absence of compound. The relief of fluorescence homoquenching of 100 nM T7C-AF TNF-α after dilution in a 200-fold excess of unlabeled TNF-α was used to monitor subunit disassociation from TNF-α trimer (solid circles). Nonlinear regression analysis using the appropriate kinetics equation (*12*) gave a calculated rate of 0.000093 s^{-1} per monomer dissociation event (solid circles). Addition of 30 μM compound to the homoquenching assay accelerates the observed time course of fluorescence increase and yields a calculated rate constant of 0.059 s^{-1} per monomer dissociation event (solid triangles). Open circles show fluorescence in the absence of added unlabeled TNF-α. Error bars represent the standard deviation of triplicate measurements.

caused about 13 additional hydrogens per TNF-α to become surface-exposed (Fig. 4C). This number compares favorably with the 11 additional exchangeable hydrogens calculated for the TNF-α dimer relative to the published TNF-α trimer structure (*12*). These results suggest that the TNF-α dimer is more prevalent than TNF-α monomer (22 expected additional exchangeable hydrogens) under these conditions.

We considered two possible models to explain how the compound acts to cause formation of TNF-α dimer. In the first, predissociation-dependent model, the compound functions passively by binding to and stabilizing the TNF-α dimer only after a TNF-α trimer subunit has spontaneously dissociated. In the second, predissociation-independent model, the compound functions actively by interacting with the TNF-α trimer to promote the dissociation of a subunit to form TNF-α dimer.

To discern which mechanism operates, we developed a sensitive fluorescence homoquenching–based assay (*16*) to examine the kinetics of subunit dissociation from the TNF-α trimer. In this assay, we monitored the decrease in fluorescein homoquenching that occurs when closely associated molecules become separated. A TNF-α mutant (T7C) having a free thiol in the region of the disordered N terminus was covalently coupled to the 5-iodoacetamidofluorescein (5-IAF) so that each subunit of TNF-α trimer was fluorescently labeled. Binding analysis of this highly fluorescent adduct reagent (called T7C-AF) showed it possessed the same affinity for TNFR1 as did unmodified wild-type TNF-α.

Addition of the T7C-AF reagent to a 200-fold excess of unlabeled TNF-α resulted in a time-dependent increase in fluorescence as dissociating T7C-AF subunits were replaced by subunits not fluorescently tagged (Fig. 4D). Fitting the resulting curves to the appropriate kinetic equation (*12*) by nonlinear regression analysis allowed us to determine the dissociation rate of monomer subunits from the TNF-α trimer to be 0.000093 s^{-1}. The addition of 30 μM compound resulted in a much more rapid subunit dissociation rate of 0.057 s^{-1}, representing a 600-fold acceleration from the spontaneous dissociation rate of TNF-α (Fig. 4D). Additionally, time course experiments using the TNFR1 enzyme-linked immunosorbent assay (ELISA) showed rapid TNF-α inactivation consistent with these measurements (fig. S3). The highly accelerated dissociation rate indicates that the compound actively promotes subunit dissociation and functions through the predissociation-independent model described previously. This finding implies that the initial step for inactivation is the binding of the compound to TNF-α trimer to form an intermediate complex that undergoes accelerated subunit dissociation.

We found evidence for compound association with intact trimer from studies done at superphysiological TNF-α concentrations. Analysis by sedimentation equilibrium of a mixture of 45 μM TNF-α and 68 μM compound was performed, and compound migration within that mixture was tracked by its absorbance at 310 nm. The compound migrated with an apparent molecular weight consistent with being bound to intact TNF-α trimer (fig. S4). Moreover, measurements by tandem gel filtration and dynamic light scattering showed that 3 to 30 μM TNF-α concentrations retained the molecular weight of trimer in the presence of 30 μM compound (table S3). Lastly, we showed that the intrinsic tryptophan fluorescence (ITF) of 0.5 to 5 μM TNF-α was 85% quenched by addition of 50 μM compound, suggesting that the compound is bound in proximity to Trp^{28} and Trp^{114} within the TNF-α trimer. Measurements of the rate of restoration of TNF-α ITF after a 10-fold dilution of this quenched complex to a concentration well below the IC_{50} of the compound showed that the effect is reversible and that the dissociation rate of the compound from the TNF-α trimer is rapid (fig. S5).

Taken together, our results indicate that the compound-associated TNF-α trimer predicted by the predissociation-independent model exists and represents a more weakly associated oligomeric form than the free TNF-α trimer. Under lower physiologically relevant TNF-α concentrations, this proposed complex is highly unstable and rapidly inactivated by subunit dissociation. However, at higher superphysiological TNF-α concentrations there is sufficient TNF-α present to stabilize compound-associated TNF-α trimer as the prevalent form at equilibrium.

The co-structure and mechanism of action of the TNF-α inhibitor described herein

demonstrates that small molecules that function by disrupting tightly preassociated oligomeric proteins are feasible. Although small-molecule inhibitors that block dimer formation exist for a number of intracellular homodimeric proteins (*10*, *17*), many may function through a predissociation-dependent mechanism. For example, an inhibitor (*18*, *19*) of inducible nitrous oxide synthase (iNOS) inhibits the intracellular association of iNOS monomers into enzymatically active iNOS dimer yet is inactive against isolated dimeric iNOS. Inhibitors of this type may have limited utility against extracellular preassembled multimeric proteins like TNF-α that have very slow spontaneous subunit dissociation rates.

In contrast, the TNF-α inhibitor we describe binds to the intact biologically active trimer and accelerates subunit dissociation to rapidly inactivate the cytokine. Interestingly, this activity together with the co-structure of the TNF-α dimer compound complex suggests that the compound is able to access the normally buried interior of TNF-α trimer. It is possible that the compound achieves this by exploiting an intrinsic dynamic breathing between the subunit interfaces that may occur in solution-phase TNF-α trimer, but the precise mechanism by which the compound functions remains to be elucidated. The results we have described should enable the design of appropriate assays that may allow for the identification of potent small-molecule inhibitors that inactivate multimeric proteins via a rapid predissociation-independent subunit dissociation process.

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14. Single-letter abbreviations for the amino acid residues are as follows: G, Gly; L, Lys; Q, Gln; S, Ser; and Y, Tyr.
15. For the two compounds (including hydrogens) within the unit cell surfaces, areas of 337 Å^2 and 315 Å^2 are calculated for an average of 326 ± 11 Å^2 of surface contact area on the TNF-α dimer.
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23. X-ray crystallographic data were deposited in the Protein Data Bank under accession code 2AZ5. Compound libraries that led to the discovery of the TNF-α inhibitor were produced at Sunesis Pharmaceuticals by A. A. Virgilio. Crystal data collection was carried out at the Stanford Synchrotron Radiation Laboratory (SSRL), a national user facility operated by Stanford University on behalf of the U.S. Department of Energy, Office of Basic Energy Sciences. The SSRL Structural Molecular Biology Program is supported by the U.S. Department of Energy, Office of Biological and Environmental Research, and by NIH, National Center for Research Resources, Biomedical Technology Program, and National Institute of General Medical Sciences.

Supporting Online Material

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Materials and Methods
Figs. S1 to S6
Tables S1 to S3

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Structure of a V3-Containing HIV-1 gp120 Core

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The third variable region (V3) of the HIV-1 gp120 envelope glycoprotein is immunodominant and contains features essential for coreceptor binding. We determined the structure of V3 in the context of an HIV-1 gp120 core complexed to the CD4 receptor and to the X5 antibody at 3.5 angstrom resolution. Binding of gp120 to cell-surface CD4 would position V3 so that its coreceptor-binding tip protrudes 30 angstroms from the core toward the target cell membrane. The extended nature and antibody accessibility of V3 explain its immunodominance. Together, the results provide a structural rationale for the role of V3 in HIV entry and neutralization.

The HIV envelope spike mediates binding to receptors and virus entry [reviewed in (*1*)]. The trimeric spike is composed of three gp120 exterior and three gp41 transmembrane envelope glycoproteins. CD4 binding to gp120 in the spike induces conformational changes that allow binding to a coreceptor, either CCR5 or CXCR4, which is required for viral entry (*2*–*6*). Snapshots of the gp120 entry mechanism have been visualized through crystal structures of unliganded and CD4-bound states (*7*, *8*). However, an essential component of the coreceptor binding site, the third variable region (V3), has been absent from previous structural characterizations of the gp120 core.

V3 typically consists of 35 amino acids (range 31 to 39) and plays a number of important biological roles [reviewed in (*9*)]. Not only is it critical for coreceptor binding, but it also determines which coreceptor, CXCR4 or CCR5, will be used for entry (*10*). In addition, V3 may interact with other elements in the viral spike to control the overall sensitivity of the virus to neutralization (*11*). Finally, immunization with HIV-1 envelope glycoproteins often elicits neutralizing responses directed primarily against V3 (*12*, *13*).

The structure of V3 in the context of core gp120 bound to CD4, described here, now reveals the entire coreceptor binding site. We propose that V3 acts as a molecular hook, not only for snaring coreceptor but also for modulating subunit associations within the viral spike. Its extended nature is compatible with the elicitation of an immunodominant antibody response.

The extreme glycosylation and conformational flexibility of gp120 inhibit crystallization. We used variational crystallization and various technologies adapted from structural genomics to obtain crystals suitable for x-ray structural analysis (*14*–*16*). Constructs of the gp120 core with V3 from three clade B isolates (HXBc2, JR-FL, and YU2) were expressed in

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Drosophila S2 cells, and the deglycosylated, purified proteins were complexed with CD4 and a CD4-induced antibody (*16*). A total of 13 different complexes were screened robotically, and crystallization hits were optimized manually. The gp120 core with V3 from JR-FL (*17*, *18*), when complexed to CD4 (two-domain) and the antigen-binding fragment (Fab) of the X5 antibody (*19*), formed hexagonal crystals that diffracted to approximately 3.5 Å resolution with x-rays provided by an Advanced Photon Source undulator beam line (SER-CAT) (table S1). The structure was solved by molecular replacement (*16*) and is shown in Fig. 1.

As expected, the overall assembly of CD4, X5, and core gp120 resembled the previously determined individual structures of CD4 (*20*, *21*) and of free X5 (*22*) as well as the complex of core gp120 bound to CD4 (*8*, *23*). For core gp120, some differences were observed in the variable loops and also at the N terminus, regions where variations in gp120 have previously been observed (*7*, *8*, *23*, *24*). Structural resemblance was maintained around the base of V3, indicating that the previous truncation (*7*, *8*, *23*, *24*) did not distort this region of the core. In X5, a large structural difference was observed for the third complementarity-determining loop of the X5 heavy chain (CDR H3). Comparison of the refined structures of free X5 (*22*) and bound X5 showed Cα movements of up to 17 Å, one of the largest induced fits observed for an antibody (fig. S1).

The gp120 envelope protein is composed of inner and outer domains, named for their expected orientation in the oligomeric viral spike (*8*). V3 emanates from neighboring staves of the stacked double barrel that makes up the outer domain; it is almost 50 Å long from the disulfide bridge at its base to its conserved tip, but is otherwise only 15 Å wide and 5 Å deep (Fig. 2). Overall, it can be subdivided into three structural regions: a conserved base, which forms an integral portion of the core; a flexible stem, which extends away from the core; and a β-hairpin tip. In the crystal structure, the flexibility and position of the V3 tip may be influenced by a lattice contact, in which hydrogen bonds are made to the exposed backbone of the V3 β ribbon between Ile^{307} and Ile^{309}. Tenuous side-chain contacts are also observed for the returning strand in the V3 stem with X5, as well as with V4 of a symmetry-related gp120 molecule, but these side-chain contacts are unlikely to influence its conformation.

Features of gp120 important for coreceptor binding have been mapped by mutagenesis to two regions: (i) the V3 tip, and (ii) the gp120 core around the bridging sheet, the V3 base, and neighboring residues (*25*–*28*). Analysis of these two regions on this new structure indicates that they are conserved in both sequence and structure (figs. S2A and S3).

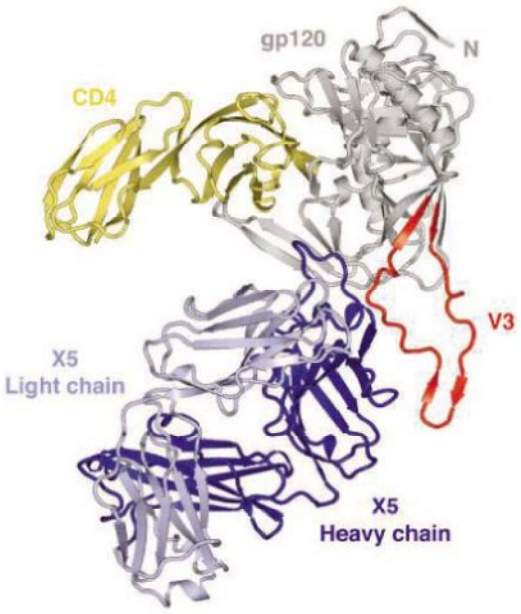

Fig. 1. Structure of an HIV-1 gp120 core with V3. The crystal structure of core gp120 (gray) with an intact V3 (red) is shown bound to the membrane-distal two domains of the CD4 receptor (yellow) and the Fab portion of the X5 antibody (dark and light blue). In this orientation, the viral membrane would be positioned toward the top of the page and the target cell toward the bottom.

The structural conservation of the V3 tip was surprising here in light of the apparent flexibility of the intervening stem, but we found the V3 tip to be strikingly similar in the context of the core, in antibody–V3 peptide complexes, and as a free peptide; such similarity is consistent with previous reports of recurring conformations for the V3 tip in antibody-peptide complexes (*29*). The structure shows that conserved regions important for coreceptor binding are separated by 10 to 20 Å and by portions of the V3 stem with moderate to high sequence variation (fig. S2).

Emerging data on the structures of the coreceptors indicate that the regions identified as being important for binding gp120 the coreceptor N terminus and the second extracellular loop may also be spatially separated (*30*). By integrating the two-site gp120 binding site on the coreceptor with the two-site coreceptor binding site that we observe in the core V3 gp120 structure, we propose that the N terminus of the coreceptor reaches up and binds to the core and V3 base while the V3 tip of gp120 reaches down to interact with the second extracellular loop of the coreceptor (Fig. 3B). Support for this model comes from several sources: (i) Biochemical studies show that the binding of CCR5 N-terminal peptides to gp120 is affected by gp120 alterations only on the core and around the base of V3 (*28*); and (ii) small-molecule inhibitors of HIV entry that bind to the second extracellular loop of the coreceptor are observed to no longer affect mutant viruses with V3 truncations (*31*).

Does binding of the V3 tip to the coreceptor initiate gp41-mediated conformational changes? Despite general tolerance of the V3 stem to changes in sequence, there is less tolerance for insertions or deletions than in other gp120 variable loops. We superimposed the core V3 structure on the modeled gp120 core trimer that we previously obtained by optimization of quantifiable surface parameters (*32*). This trimeric model orients gp120 in the context of both cell-surface CD4 and the target cell membrane. Such a superposition projects the highly conserved Pro-Gly of the V3 tip 30 Å toward the target cell membrane (Fig. 3A).

Different coreceptors, primarily CXCR4 or CCR5, can support HIV-1 entry. Sequence analysis has defined an 11/25 rule: If the 11th or 25th positions of V3 are positively charged, viruses will use CXCR4; otherwise they use CCR5 (*33*). In addition, V3 sequences are more conserved for CCR5-using viruses (fig. S2). The structure shows that positions 11 and 25 (residues 306 and 322) are within the variable stem. They each project about the same distance away from the core but are separated by a Cα distance of 17 Å (fig. S2). This separation suggests that positions 11 and 25 recognize different portions of the coreceptor.

CD4 induces large conformational changes in gp120. Before CD4 binding, V3 may not protrude precisely as observed here for the CD4-triggered coreceptor binding state of gp120 (*3*, *34*). However, structural comparison of unliganded versus CD4-bound conformations of gp120 (*7*, *8*) reveals that the local conformation of the region of the outer domain from which V3 emanates is mostly unchanged. Thus, the extended structure of V3 that we observe here should be generally representative of V3.

Immunization with gp120 or gp120/gp41 in various contexts may elicit an immune response in which virtually all of the neutralizing activity

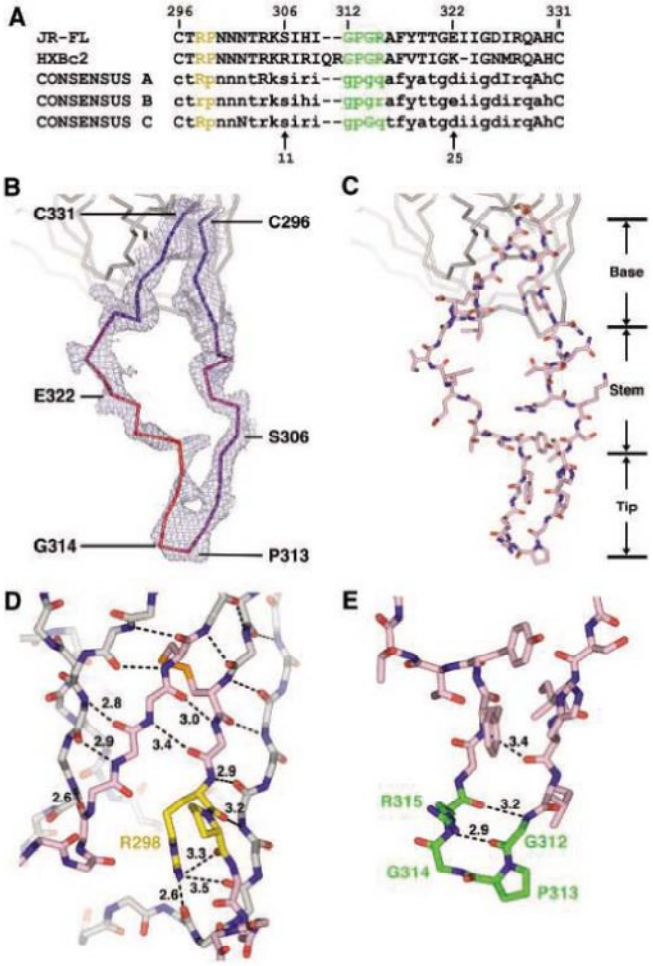

Fig. 2. V3 sequence and structure. (**A**) V3 sequence. The sequences of JR-FL (*17*) and HXBc2 are shown along with the consensus sequence of clades A, B, and C. For the consensus sequences, absolutely conserved residues are shown in uppercase, with variable residues in lowercase (*37*). Single-letter amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr. The conserved (Arg-Pro) and (Gly-Pro-Gly-Arg) motifs are colored yellow and green, respectively, and are highlighted with the same colors in (D) and (E). (**B**) V3 electron density and *B* values. $2F_{obs} - F_{calc}$ density is shown for the entire V3 region and contoured at 1σ. V3 is color-coded by *B* value from blue (lower atomic mobility) to red (higher mobility). (**C**) V3 structure. The entire V3 is shown (color code: salmon, carbon atoms; red, oxygen atoms; dark blue, nitrogen atoms; orange, disulfide bond). Regions corresponding to the fixed base, accordion-like stem, and β-hairpin tip are labeled. (**D**) Close-up view of the V3 base. From its N terminus (Cys^{296}), V3 extends the antiparallel sheet on the outer domain of gp120. After hydrogen bonding for three residues, additional sheet contacts are interrupted by two conserved residues: Arg^{298}, whose side-chain hydrogen bonds to three carbonyl oxygens, including two on the neighboring outer domain strand; and Pro^{299}, which initiates the separation of outgoing and returning V3 strands. In the returning strand, antiparallel β-sheet interactions with core gp120 recommence with the carbonyl of residue 297 and continue to the disulfide at Cys^{331}. Main-chain atoms are shown for the core and V3 base, colored the same as in (C). Hydrogen bonds are depicted with dashed lines, with select distances in Å. All atoms of the highly conserved Arg^{298}, Pro^{299}, and Cys^{296}-Cys^{331} disulfide are shown, with Arg and Pro carbons highlighted in yellow and disulfide in orange. (**E**) Conformation of the V3 tip. From Ser^{306} to Gly^{312}, the main chain assumes a standard β-conformation, which terminates in a Gly-Pro-Gly-Arg β-turn (residues 312 to 315) (*29*, *38*). After the turn, the returning density is less well defined, indicative of some disorder. All atoms of the tip are colored as in (C), with carbon atoms of the conserved tip highlighted in green. Hydrogen bonds that stabilize the β hairpin are shown as in (D).

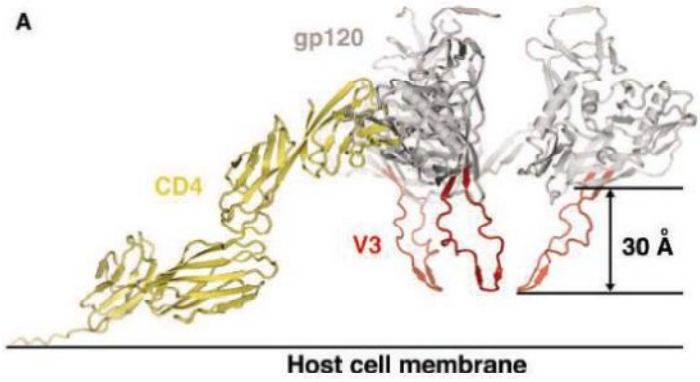

Fig. 3. Modeled trimer and co-receptor schematic. (**A**) V3 in the context of a trimer at the target cell surface. The structure of the CD4-triggered gp120 with V3 was superimposed onto the structure of four-domain CD4 (*39*) and the trimer model obtained by quantification of surface parameters (*32*). In this orientation, the target cell membrane and coreceptor are expected to be positioned toward the bottom of the page. (**B**) Schematic of coreceptor interaction. CCR5 (green) is shown with its tyrosine-sulfated N terminus (at residues 3, 10, 14, and 15) and three extracellular loops (ECLs). V3 (red) is shown with its conserved base interacting with the sulfated CCR5 N terminus and its flexible legs allowing its conserved V3 tip to reach the second ECL of CCR5.

is directed at V3. We examined the crystal and nuclear magnetic resonance structures of V3-reactive antibody-peptide complexes for clues to this immunodominant response (fig. S3). Although the conformation of V3 peptides in these antibody-peptide complexes varies somewhat, the Pro-Gly tip is more conserved. Superimposing the conserved tip in the peptides with the V3 tip in the core V3 structure permits the V3 peptide-binding antibodies to be placed in the context of the gp120 core. The antibodies completely surround V3 (Fig. 4). Although the accessibility of V3 may be quite different on a primary isolate in its pre-CD4 trimeric state, the extended nature of V3 observed here, when coupled to mechanisms that cloak the rest of the HIV envelope from antibody binding (*1*, *35*, *36*), is consistent with its ability to generate an immunodominant response.

The attributes that we observe for V3 (i.e., high relative surface area, chemically reactive backbone, conformational flexibility, and overall extended nature) may allow V3 to serve as a general molecular hook. Before CD4 binding,

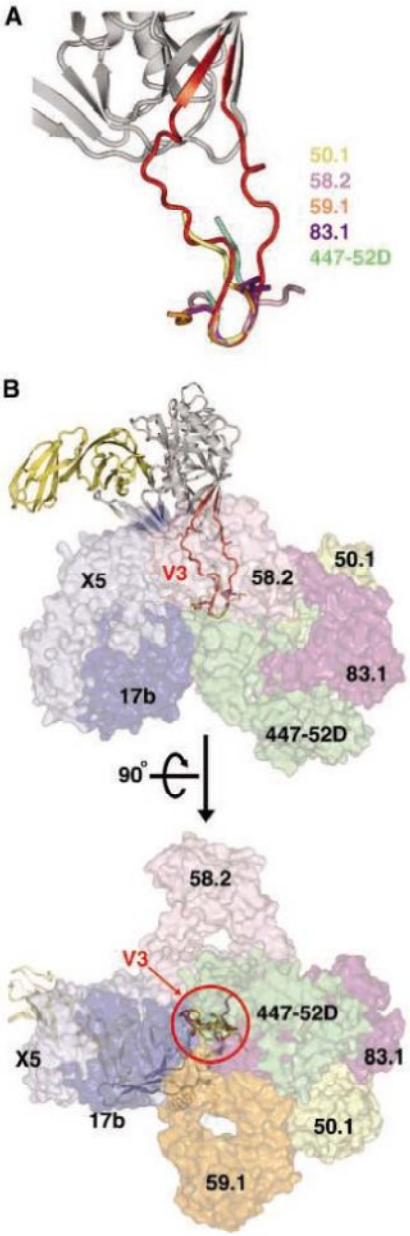

Fig. 4. Accessibility of V3 to neutralizing antibodies. The molecular surfaces of neutralizing antibodies that block coreceptor binding are shown superimposed onto gp120 in the context of V3; antibodies 17b and X5 bind to the conserved coreceptor binding site on the core, whereas monoclonal antibodies 50.1, 58.2, 59.1, 83.1, and 447-52D bind to V3. (**A**) Superposition of V3 structures. Core with V3 is shown with V3 peptides as extracted from peptide–anti-V3 neutralizing antibody complexes after superposition of the conserved V3 tip. (**B**) Antibody accessibility of V3. Core gp120 with V3 (ribbon representation) is shown in two perpendicular views with Fab fragments (molecular surface representation) of antibodies that bind at the coreceptor binding site on either core or V3. V3 is completely surrounded by neutralizing antibodies, suggesting a high degree of accessibility for generating an immune response.

these attributes would enhance the ability of V3 to grasp neighboring protomers on the viral spike. Such quaternary interactions would explain V3's influence on overall neutralization sensitivity for example, its ability to transfer neutralization resistance from YU2 to HXBc2 (*11*). After CD4 binding, the coreceptor binding site forms and V3 would jut prominently toward the target cell membrane. In this context, binding at the V3 tip may act as a "ripcord" to initiate gp41-mediated fusion. Our results provide a context for coreceptor interactions and suggest how V3, by altering quaternary interactions, can influence HIV evasion of the immune system and also trigger HIV entry into cells. The structure itself represents an elegant evolutionarily malleable solution that balances competing requirements of functional conservation and antigenic variation.

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Supporting Online Material

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Species Loss and Aboveground Carbon Storage in a Tropical Forest

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Tropical forest biodiversity is declining, but the resulting effects on key ecosystem services, such as carbon storage and sequestration, remain unknown. We assessed the influence of the loss of tropical tree species on carbon storage by simulating 18 possible extinction scenarios within a well-studied 50-hectare tropical forest plot in Panama, which contains 227 tree species. Among extinction scenarios, aboveground carbon stocks varied by more than 600%, and biological insurance varied by more than 400%. These results indicate that future carbon storage in tropical forests will be influenced strongly by future species composition.

In terrestrial ecosystems, functional diversity and relative abundance influence both the magnitude (*1*–*5*) and variability (*6*) of aboveground biomass. Aboveground biomass, in turn, substantially determines an ecosystem's potential for carbon storage, which plays an important role in the regulation of atmospheric CO_2 and global climate change (*7*, *8*). Biodiversity, however, is changing rapidly in response to a variety of anthropogenic drivers (*9*–*13*). The potential for terrestrial carbon sequestration could be altered sharply by ensuing changes in species composition.

The relationship between diversity and aboveground biomass has been examined in herbaceous ecosystems such as grasslands (*3*, *6*), meadows (*14*), and wetlands (*15*). These ecosystems, however, account for just 16% of the estimated 558 Pg (1 Pg = 10^{15} g) of carbon stored in vegetation (*16*). The remaining 470 Pg of carbon reside in forests, woodlands, and savannahs, more than half (54%) of which are tropical.

In tropical forests, conventional biodiversity manipulations are prohibitively costly because of the large number of tree species as well as the size and longevity of tropical trees. Instead, we simulated species extinctions in a diverse tropical forest by using data from the 50-ha Forest Dynamics Plot on Barro Colorado Island (BCI), Panama (*17*). Our model, which expanded on the approach of Solan and colleagues (*18*), enabled us to establish many species combinations and compositions under different extinction scenarios to explore the realm of possible futures for aboveground carbon storage. We simulated these effects on aboveground biomass by removing species with a probability proportional to extinction-related traits (e.g., small population size) and replacing the eliminated basal area with a random draw from the remaining community (Fig. 1) (*19*). We used functional traits [wood density and volume per unit basal area; see equation S1 in (*19*)] to quantify the aboveground carbon pool for each simulated community. Thus, variation in functional diversity (the diversity of these functional traits among species) governs ecosystem response.

We explored three classes of trait-based extinction scenarios that represent a broad spectrum of extinction mechanisms (table S1). One class consists of extinction associated with population traits such as low population growth rates, low densities, and endemism, which are known correlates of extinction risk (*20*–*22*). The second class consists of extinction scenarios related to management or harvest strategies, such as selective harvest for hardwoods or harvesting the most common or the largest trees; this scenario uses related traits such as wood density, stature, and abundance. The third class consists of species' responses to environmental change, such as changes in precipitation, rates of disturbance, or elevated CO_2 (*23*, *24*). We also included a random extinction scenario that serves as a reference and reflects the approach commonly used in combinatorial biodiversity experiments.

The extinction scenarios produced divergent effects in both the magnitude and variability of aboveground carbon storage (Fig. 2 and table S1). For instance, the extinction of species with the lowest wood density led to strong increases (+75%) in carbon storage (Fig. 2, G to I, and table S1), whereas the loss of species that attain large stature resulted in a strong decline in carbon stocks (Fig. 2, D to F, and table S1). Extinction scenarios demonstrated different degrees of loss of biological insurance (i.e., decreasing predictability or increasing variability as species are lost) (Fig. 2). For instance, the loss of endemic species resulted in relatively less loss of biological insurance, largely because endemics tend to be locally rare and contribute little to total carbon stocks (Fig. 2, S to U, and table S1). These differences in loss of biological insurance are attributable to the extent of variability in functional traits, which is strongly dependent on species identity and community composition.

Anthropogenic effects on tropical forest diversity vary widely. Selective logging typically removes a small number of species from a community (*25*), whereas conversion to forest plantations removes all but one or two species. Our results show that selective logging for

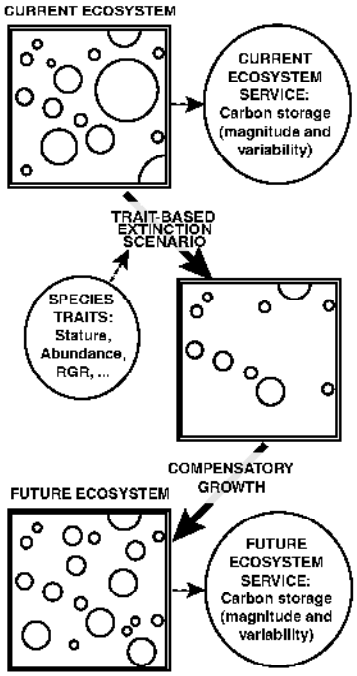

Fig. 1. Impact of tree-species extinctions on carbon storage within an extant tropical forest. Ellipses represent input and output variables, solid arrows represent process steps, and squares represent the three states of the BCI 50-ha forest plot, in which circles represent trees of different diameter and species identity. On the basis of the relationship between known composition and relative abundance of trees in the 50-ha plot and current aboveground carbon storage (**top**), future carbon storage (**bottom**) can be estimated under different extinction scenarios. Extinction scenarios use trait-based responses to environmental change (e.g., habitat fragmentation and elevated CO_2). The middle square represents the transitory state in which extinction has led to reduced abundance. After compensatory growth that replaces basal area lost to extinction, plant traits are used to estimate the ecosystem service of a less diverse forest. RGR, relative growth rate.

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species with high wood density, large diameter, high basal area, or maximal wood volume will likely lead to overall declines in carbon storage of 70, 29, 17, and 21%, respectively (Fig. 2 and table S1). In contrast, conversion to plantations that use species with high wood density may increase aboveground carbon storage by up to 75% if never harvested (Fig. 2, G to I, and table S1). However, conversion to plantation may cause decreases in belowground carbon and reduce other ecosystem services such as fruit production or water quality.

In addition to direct anthropogenic forces, changes in forest composition have been observed in natural forests of the Amazon, where increased stem turnover and liana abundance may favor fast-growing species (*24*, *26*, *27*). Our results suggest that if this trend persists, a shift toward fast-growing species in tropical forests could lead to a 34% decrease in carbon storage (Fig. 2, M to O, and table S1). Climate observations and model predictions (*28*, *29*) both suggest continued decreases in precipitation over much of the humid tropics. Our model predicts a slight increase in carbon stocks (10%), with a shift toward drought-tolerant species, and notably, a 48% increase in the loss of biological insurance relative to random extinction (Fig. 2, P to R, and table S1). Thus, species diversity may provide increased biological insurance in the face of species loss due to reduced precipitation.

Our results should not be interpreted as specific predictions for future carbon storage but rather as an assessment of the relative effects of nonrandom species losses. Drivers of biodiversity change will likely alter additional mechanisms that regulate carbon storage. For example, disturbances caused by selective logging decrease carbon storage in the short term, whereas increased precipitation may increase carbon storage through effects on net primary productivity. In addition, because we based our species-poor communities on the observed composition and basal area of the BCI plot, the retained species maintain their relative abundance and size-frequency distributions within these impoverished communities. For this reason, to the extent that complementarity, facilitation, and sampling effects (*30*–*32*) occur in the intact 50-ha plot, these forces have equivalent effects in our simulated communities and are invariant

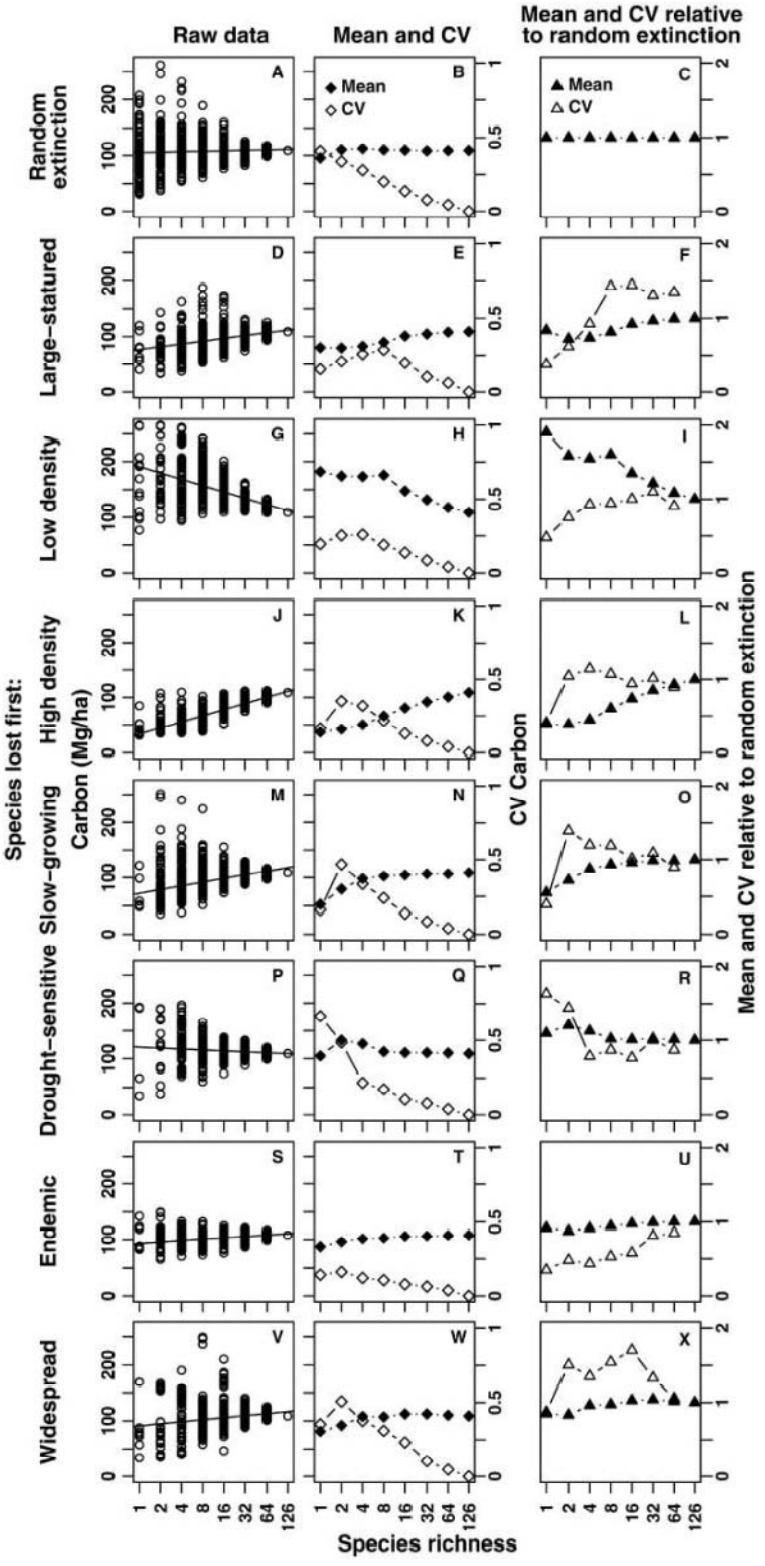

Fig. 2. Representative results of simulated influences of biodiversity on aboveground carbon storage in the 50-ha Forest Dynamics Plot on BCI, Panama. The intact community included 126 species; the *x* axes have a $\log_2$ scale. The left panels show simulation results (open circles) and linear fit (solid line) of the effect of $\log_2$ species richness on aboveground carbon storage. The center panels show the mean (solid diamonds) and coefficient of variation (CV) (open diamonds) of carbon storage. The right panels show the mean (solid triangles) and CV (open triangles) of carbon storage relative to random extinction. (**A** to **C**) Random extinction. (**D** to **F**) Large-statured species lost first. (**G** to **I**) Species with low wood density lost first. (**J** to **L**) Species with high wood density lost first. (**M** to **O**) Slow-growing species lost first. (**P** to **R**) Drought-sensitive species lost first. (**S** to **U**) Endemics lost first. (**V** to **X**) Widespread species lost first. Values are lower than those reported elsewhere because we excluded 101 species (21% of aboveground carbon) for which we lacked wood-density data.

with species richness. If complementarity, facilitation, and sampling effects do contribute to positive effects of diversity on carbon storage on BCI, as often has been observed in simpler communities (*1*–*5*), then actual carbon storage in species-poor communities may be lower than our models predict. Indeed, high diversity within the BCI plot may reduce losses of carbon to density-dependent effects of herbivores and pathogens (*33*, *34*).

Species extinctions are rarely random but rather are driven by the interaction between species traits and environmental change. Our results show that tropical forest carbon storage depends on species composition and on the mode and manner in which species are lost. By extension, carbon storage in reforested landscapes depends especially on the functional diversity of the available species pool. Because variability decreases with species richness, and because extinction scenarios differ widely in magnitude and direction, management options that favor high diversity will maximize predictability for tropical forest carbon storage and sequestration.

We have examined only one of many ecosystem services provided by tropical forests. Extinction scenarios that maximize carbon storage may minimize other services such as flood protection, nutrient retention, cultural services, pollination, biological control, and provisioning of fruits, nuts, and bush meat (*10*). Human domination of terrestrial and aquatic landscapes has made us increasingly dependent on a reduced number of species to provide critical ecosystem services. Given uncertainty in both the nature of extinction and the variety of ecosystem services required for human well-being, we may best be able to meet these demands by maximizing the pool of species on which we depend.

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The Pseudo-Response Regulator *Ppd-H1* Provides Adaptation to Photoperiod in Barley

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Plants commonly use photoperiod (day length) to control the timing of flowering during the year, and variation in photoperiod response has been selected in many crops to provide adaptation to different environments and farming practices. Positional cloning identified *Ppd-H1*, the major determinant of barley photoperiod response, as a pseudo-response regulator, a class of genes involved in circadian clock function. Reduced photoperiod responsiveness of the *ppd-H1* mutant, which is highly advantageous in spring-sown varieties, is explained by altered circadian expression of the photoperiod pathway gene *CONSTANS* and reduced expression of its downstream target, *FT*, a key regulator of flowering.

Plants have evolved sophisticated controls to ensure that flowering occurs when there is the greatest chance of pollination, seed development, and seed dispersal. Usually this involves restricting flowering to a specific time of year. To achieve this, many plants use photoperiod as an environmental cue to regulate development. The timing of flowering has important impacts on crop yield, and the modification of responses to environmental cues by human selection has been central to the success and spread of agriculture.

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The control of flowering by photoperiod is understood best in the long-day (LD) dicot *Arabidopsis* and the short-day (SD) monocot cereal rice. In *Arabidopsis*, expression of *GIGANTEA* (*GI*) and *CONSTANS* (*CO*) is regulated by the circadian clock such that coincidence of the *CO* expression peak with light only occurs in LD conditions. Light-stabilized CO protein is a transcription factor inducing downstream genes, including *FLOWERING LOCUS T* (*FT*) (*1*, *2*).

In rice, analyses of natural variation showed that *Heading date1* (*Hd1*), a major determinant of photoperiod response, is an ortholog of *CO* (*3*), that *Hd3a* is an ortholog of *FT* (*4*), and that *GI* is also conserved (*5*). However, the interaction of *Hd1* with *FT* is altered such that *FT* expression is inhibited in LDs (*2*, *5*). The rice *Ehd1* gene also controls photoperiod response but has no direct counterpart in *Arabidopsis* and regulates *FT* independently of *Hd1* (*6*). Photoperiod response in rice therefore has conserved and novel aspects compared with *Arabidopsis*, but in both species increased *FT* expression is crucial to the induction of flowering. Genes controlling photoperiod response in temperate cereals such as barley (*Hordeum vulgare*) have not been identified previously.

Barley varieties can be broadly classified as winter or spring types. Winter (fall-sown) bar-

leys require vernalization and usually show strong promotion of flowering in response to LDs. This is typical of *H. spontaneum*, the wild progenitor of barley, suggesting that this is the ancestral condition. Spring (spring-sown) barleys lack vernalization requirement and show weak or strong response to LDs depending on whether they have been selected for long or short growing seasons, respectively. In long growing seasons, as in Western Europe and much of North America, reduced response to photoperiod allows spring-sown plants to extend the period of vegetative growth and accumulate additional biomass that supports higher yields.

The major determinant of LD response in barley is the *Photoperiod-H1* (*Ppd-H1*) locus (*7*, *8*). The late-flowering *ppd-H1* allele is recessive (Fig. 1A), suggesting that reduced response results from a mutation that impairs gene function. *Ppd-H1* does not correspond to either of the barley *CO*-like genes (*HvCO1* and *HvCO2*) (*9*), showing that different major determinants of photoperiod adaptation have been selected in barley and rice.

We identified *Ppd-H1* by positional cloning, using colinearity of the barley *Ppd-H1* region with rice and *Brachypodium* (*10*). Fine-scale mapping using lines derived from an Igri (*Ppd-H1*) and Triumph (*ppd-H1*) cross (Fig. 1, B and C) enabled a physical map of the *Ppd-H1* region to be developed (Fig. 1D). Recombinants defined a region containing a single gene that was a pseudo-response regulator (*PRR*) most similar overall to *Arabidopsis* PRR7 (fig. S1). PRR proteins are characterized by two conserved regions, a pseudoreceiver domain with similarities to bacterial two-component signaling systems and a CO, CO-like, and TOC1 (CCT) domain that is also found in the CO family (*11*). The barley *PRR* gene was amplified by polymerase chain reaction (PCR) from Igri and two *H. spontaneum* accessions (JIC-1894 and JIC-1947) crossed with Igri and shown to have the *Ppd-H1* allele. Morex, which provided the bacteria artificial chromosome (BAC) sequence, was crossed with Igri and shown to have the *ppd-H1* allele. Other *ppd-H1* lines sequenced were Triumph, Golden Promise, and Optic. This revealed 23 polymorphisms, of which 7 were single nucleotide polymorphisms (SNPs) that produced amino acid changes distinguishing *Ppd-H1* and *ppd-H1* alleles (1, 12, 15, 20, 21, 22, and 23 in Fig. 1E). Regions containing these SNPs were sequenced from a further eight *H. spontaneum* accessions known to be early flowering in LDs and nine barley varieties previously classified as early or late flowering in LDs (table S3). In the extended set, four SNPs (1, 15, 22, and 23) remained completely associated with *Ppd-H1* or *ppd-H1* alleles (Fig. 2). Three were in regions of low conservation with rice and *Arabidopsis* (fig. S1), but the fourth produced a Gly-to-Trp change in the CCT domain affecting a residue that is conserved in all CCT domain genes identified to date (fig. S2) and that is the most likely causal basis of the *ppd-H1* mutation. The CCT domain mutation was a G-to-T change, which removed a *Bst*UI restriction site, providing a simple PCR-based assay for the *ppd-H1* allele (fig. S3).

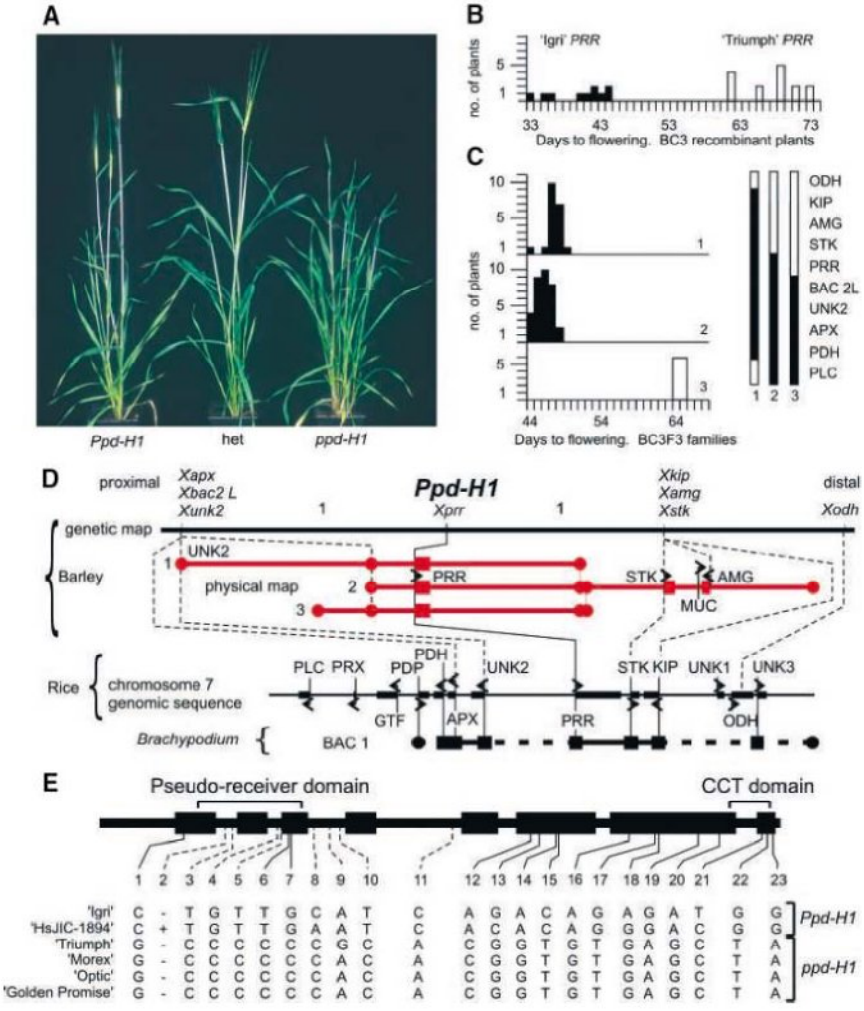

Fig. 1. Flowering phenotypes, genetic and physical mapping of the *Ppd-H1* locus, and sequence variation between alleles. **(A)** Phenotypes of homozygous *Ppd-H1* (left), heterozygous *Ppd-H1*/*ppd-H1* (middle), and homozygous *ppd-H1* (right) plants. **(B)** Flowering time (days to awn emergence) of BC_3 (backcross 3) recombinant plants. **(C)** Flowering time of selected families with their respective homozygous recombinant chromosomes (right) where black segments have Igri alleles and white segments have Triumph alleles. **(D)** Genetic and physical maps of the *Ppd-H1* region in barley and colinear regions in rice and *Brachypodium*. The barley genetic map has its basis in 2336 Igri × Triumph BC_3 plants and shows the numbers of recombinants in the intervals flanking the *Ppd-H1* locus. Key barley BAC clones are drawn to the same scale as the rice genomic sequence. Circles are BAC end sequences. BAC 2 was completely sequenced (AY943294). Rice chromosome 7 genomic sequence (AP005199) has annotated genes (listed in table S1) as black rectangles. The *Brachypodium* BAC shows gene content, with the solid line indicating genes with confirmed order and orientation. **(E)** Structure of *Ppd-H1* (the eight exons are shown as black rectangles) and positions of the 23 polymorphisms identified in fully sequenced *Ppd-H1* and *ppd-H1* alleles. 1 and 3 to 23 were SNPs, whereas 2 was a 5-base pair (bp) insertion/deletion polymorphism (indel). Polymorphisms in exons are indicated by solid lines.

Arabidopsis prr7 mutants showed delayed flowering in LDs but showed no significant effect in SDs (*12*, *13*), similar to the effect of *ppd-H1* (*7*, *8*). *prr7* mutants also lengthen the period of clock-mediated leaf movement (*14*) and affect the expression of clock components *CCA1* and *LHY*, implicating the gene in the phasing of the clock in relation to light (*15*, *16*). These results suggested that *ppd-H1* might affect flowering by altering the expression of photoperiod pathway genes that have circadian control. To test this, we compared gene expression in Triumph (*ppd-H1*) with a Tri-

	SNP 1	SNP 12	SNP 15	SNP 19	SNP 20	SNP 22	SNP 23	
	H		**P**			**G**	**A**	
'Igri'	C	A	C	G	A	G	G	
'Dairokkaku'	C	C	C	G	G	G	G	
'Funza'	C	C	C	G	G	G	G	
'Hayakiso'	C	C	C	A	G	G	G	
'Haruna Nijo'	C	C	C	G	G	G	G	
'Nigrinudum'	C	C	C	G	G	G	G	
'Steptoe'	C	C	C	G	G	G	G	
HsJIC-1894	C	A	C	G	A	G	G	*Ppd-H1*
HsJIC-1947	C	C	C	G	G	G	G	
HsJIC-16	C	C	C	G	G	G	G	
HsJIC-52	C	C	C	G	G	G	G	
HsJIC-144	C	C	C	G	G	G	G	
HsJIC-164	C	C	C	G	-	G	G	
HsJIC-209	C	C	C	G	G	G	G	
HsJIC-1284	C	C	C	G	G	G	G	
HsJIC-1377	C	C	C	G	G	G	G	
HsJIC-2602	C	C	C	G	G	G	G	
'Triumph'	G	C	T	A	G	T	A	
'Morex'	G	C	T	A	G	T	A	
'Barke'	G	C	T	A	G	T	A	
'Blenheim'	G	C	T	A	G	T	A	*ppd-H1*
'Kym'	G	C	T	G	G	T	A	
'Golden Promise'	G	C	T	A	G	T	A	
'Optic'	G	C	T	A	G	T	A	
	Q		**S**			**W**	**T**	

Fig. 2. Genotypes of seven barley varieties and 10 *H. spontaneum* accessions carrying the *Ppd-H1* allele and seven barley varieties carrying the *ppd-H1* allele at the seven SNPs that produce amino acid changes in the predicted protein. Polymorphism positions are shown in Fig. 1E. HsJIC-164 has a 9-bp deletion spanning SNP20. Amino acids that distinguish the alleles are shown above and below in bold: A, Ala; G, Gly; H, His; P, Pro; Q, Gln; S, Ser; T, Thr; and W, Trp.

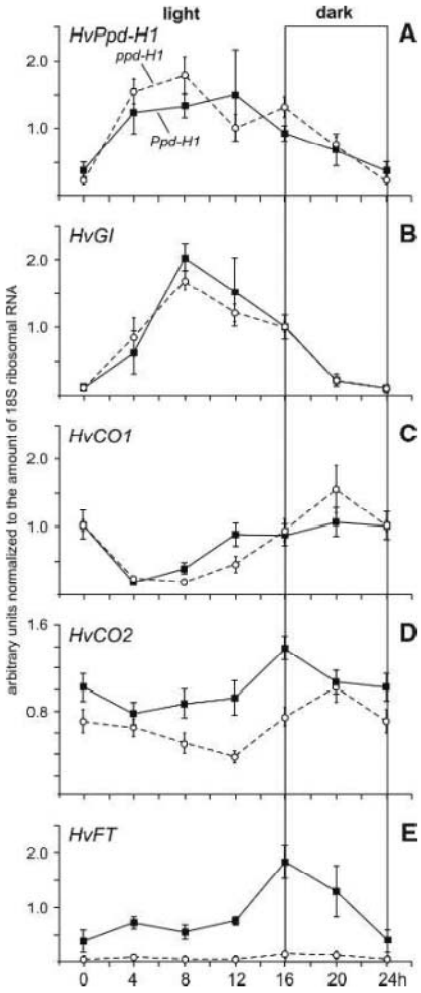

Fig. 3. Gene expression patterns in *Ppd-H1* (■, solid line) and *ppd-H1* (○, dashed line) plants grown in LD (16 hours of light) conditions and sampled at 4-hour intervals over a 24-hour period: (**A**) *HvPpd-H1*, (**B**) *HvGI*, (**C**) *HvCO1*, (**D**) *HvCO2*, and (**E**) *HvFT*. Means and standard deviations from three independent experiments are shown expressed in arbitrary units normalized against the amount of 18S rRNA (*10*). Primers and primer positions are given in table S4. Error bars indicate SEM.

umph line into which the *Ppd-H1* allele from Igri had been introgressed.

In LDs *Ppd-H1* was expressed predominantly in the early part of the day (Fig. 3A), similar to the expression patterns of *Arabidopsis PRR7* and related genes in rice. An entrainment experiment confirmed that the barley gene was under circadian control, as previously shown for *Arabidopsis* and rice *PRR* genes (*17*, *18*). Although *PRR* genes are implicated in clock function we detected no significant difference between *Ppd-H1* and *ppd-H1* plants in the expression of *Ppd-H1* itself or the barley homolog of *GI* (*HvGI*) (Fig. 3B). However, two barley *CO*-like genes (*HvCO1* and *HvCO2*) were affected. *ppd-H1* plants showed reduced expression of *HvCO1* at 8 and 12 hours (Fig. 3C), and *HvCO2* was more significantly affected with reduced expression throughout the light period and a delay in the expression peak of about 4 hours (Fig. 3D). By analogy with *Arabidopsis*, the reduced expression of *HvCO1* and *HvCO2* during the latter part of the light period in *ppd-H1* plants should reduce *FT* expression. We first tested whether barley *CO* genes behaved like *CO* in *Arabidopsis* by analyzing their expression under SDs [8 hours of light (fig. S4)]. *HvCO2* expression was lower at the start of the day but peaked at a similar time in SD and LD, whereas *HvCO1* peaked at 20 hours in SDs. The later peak of *HvCO1* expression in SDs and the higher expression of both genes at dawn in LDs were similar to CO in *Arabidopsis* (*19*). We then isolated a barley *FT* (*HvFT*) gene that is orthologous to rice *Hd3a* (*10*). Expression of *HvFT* was consistently very low in SDs (figs. S4 and S5) and was markedly lower in *ppd-H1* in LDs (Fig. 3E). The late-flowering phenotype of *ppd-H1* can therefore be explained through known photoperiod mechanisms by a reduction in *FT* expression resulting from altered circadian timing of *CO* expression. The lack of effect on *HvGI* expression suggests that the *ppd-H1* mutation does not have a strong disruptive effect on clock function or that the barley mutation affects an output linking the circadian clock to the *HvCO* genes. However, additional effects such as a direct role in *HvFT* expression cannot be ruled out.

Previous work (*14*) surveying 150 *Arabidopsis* accessions identified *PRR* genes as candidates for quantitative trait loci that provide adaptive variation by modulating circadian timing. Clock period length was correlated with latitude of origin, suggesting that these genes provide adaptive variation in photoperiod response. The identification of *Ppd-H1* as a *PRR* gene shows that the *PRR* family is of general importance for adaptation to natural and agricultural settings. Notably, comparative mapping shows that the major wheat photoperiod response genes are in colinear regions on the group 2 chromosomes (*20*) and that *Hd2* is in the colinear region of rice chromosome 7 (*21*), making these attractive targets for further analysis. The availability of *Ppd-H1* will provide greater understanding of the ways in which cereal development is regulated by environmental cues, allowing plant breeders to tailor crops to specific environments and to adjust varieties to new conditions arising from climate change.

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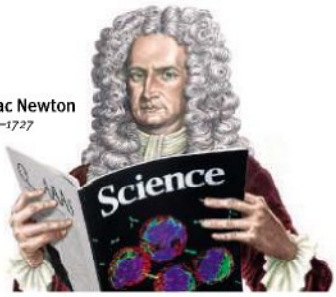

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diversity

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Research moves ahead through innovation, which can be triggered by diverse perspectives. Consequently, academic, industrial, and government institutions work to attract people from a range of cultural, disciplinary, ethnic, and gender backgrounds as well as scientists with disabilities. The experts interviewed here assess the state of diversity in science and discuss ways to improve it. BY MIKE MAY

Science thrives on diversity. Systems biology, for example, arose from a collection of disciplines once thought disparate. Such diverse interactions also arise in other fields. According to Gibor Basri, professor of astronomy at the University of California, Berkeley, "The diversity of people who work on problems is tremendous in the sense that there's a very international component to it, especially in astronomy." He adds, however, "The faculty in the U.S. in very nondiverse, mostly older, white males. It's a real issue of concern for us."

In 2005, Donna J. Nelson and Diana C. Rogers, both of the University of Oklahoma, reported that faculties include fewer women even in areas of study where women earn more Ph.D.'s than men. For example, Nelson and Rogers found that women make up only 3 percent to 15 percent of the faculty at top institutions. That means that undergraduates might never have a female professor. Moreover, broad advances in science depend on participation from all backgrounds. CONTINUED »

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Our goal is to discover and develop novel disease modifying therapies, as well as effective pain medication, for osteoarthritis and we have key openings within those ambitious initiatives. We currently have the following opportunities available in our state-of-the-art facility, located in Collegeville, PA, just a short distance from Philadelphia.

Manager/Associate Director, Osteoarthritis Research

In this role, you will provide leadership and direction for a musculoskeletal-oriented drug discovery team, including biologists and scientists within a matrix environment. You will also be responsible for applying basic biology to the drug discovery process, including innovation in animal model and assay development.

To qualify, the candidate must have a Ph.D. with postdoctoral experience in cell biology, molecular biology or pharmacology. At least 5 years' industry experience in drug discovery preferred. Requires the ability to provide leadership within a multidisciplinary team; and mastery of the application of scientific insight to establish priorities, define strategy, and implement plans to resource multiple programs appropriately. A thorough understanding of the pathophysiology of osteoarthritis and/or osteoporosis is strongly desired, as is hands-on experience with preclinical models of osteoarthritis, or in-vitro models of chondrocyte biology.
(Req. ID 27699)

Associate Scientist, Osteoarthritis Research

In this role, you will support existing drug discovery programs and new target identification for osteoarthritis, chiefly by developing innovative assays and performing compound screening. This includes the planning and execution of cartilage/chondrocyte biology experiments, as well as effective storage, interpretation and presentation of data/results.

To qualify, one must possess a BS/MS in cell biology, molecular biology (or related discipline) along with 1-5 years of experience in cell/tissue based assays and compound screening. Experience with mammalian cell culture is essential, while the use of in-vitro/ex-vivo models of cartilage/chondrocyte is highly preferred. Successful candidates should possess superior communication and data analysis software skills. Fluency with state-of-the-art molecular technology, such as RNAi, overexpression, and viral transduction is preferred. Specific knowledge of osteoarthritis and cartilage/chondrocyte biology is desired, as is a background in drug discovery.
(Req. ID 28123)

GlaxoSmithKline is dedicated to supporting you with career-long opportunities and learning. We offer a competitive benefits and compensation package designed to attract and retain the very best. ***For confidential consideration and efficient processing of your resume, please visit our website at www.gsk.com/careers referencing Req. ID of the position of interest. Indicating Req. ID is essential to search.*** *Principals only, no agencies, please.*

GSK is proud to promote an open culture, encouraging people to be themselves and giving their ideas a chance to flourish. GSK is an equal opportunity employer.

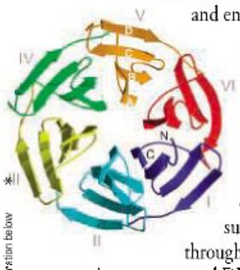
Together we can make life better.

gsk GlaxoSmithKline

Life Sciences at Missouri

Outstanding Training for Careers in Science

Interdisciplinary collaboration is the cornerstone of life sciences research at the University of Missouri-Columbia (MU). Our research credentials are stellar: MU is a member of the highly selective Association of American Universities, is classified as Doctoral/Research Intensive by the Carnegie Foundation — and MU faculty are recognized internationally for research leadership in human and animal health, food, agriculture and environmental issues.



See information below

Research facilities at MU are outstanding and include the new 200,000 square foot Life Sciences Center, which serves as a hub for state of the art science research and education for the campus community. Centralized support services are available for high throughput genomics and proteomics analyses, automated DNA sequencing and DNA microarrays, multifoton confocal microscopy, scanning and transmission electron microscopy, small animal imaging with MRI and CT, monoclonal antibody production and cell sorting, transgenic and knockout mice and rats, transgenic plants, X-ray crystallography and NMR spectroscopy. The campus is also home to the nation's largest university research reactor.

Commitment to Student Success

Recent PhD graduate Latisha Love-Gregory says it best: "The collaborative spirit of the science community at MU and the interdisciplinary approach to scientific investigation provided me an excellent training environment with great research opportunities. My faculty mentors treated me with respect and their commitment to my success was obvious. The faculty also maintained an open-door policy allowing for impromptu discussions. A "survival course" was presented for new graduate students that emphasized putting together and presenting effective scientific presentations and manuscripts. I felt ready and well-prepared for the new challenges I've encountered in my postdoctoral endeavors because of my experiences at MU."

A Great Place to Live . . .

MU is located in the city of Columbia — a vibrant, well-educated community of diverse cultures. Columbia is conveniently located in the center of the I-70 corridor through Missouri — just 2 hours from Kansas City or St. Louis — and is consistently ranked as one of the nation's most livable cities. Missouri is a beautiful state with recreational opportunities including the sophistication of big cities, the charm of small towns, and outdoor favorites — hiking and biking trails, lush forests and the clear waters of the Ozark National Scenic Riverways.

*Ribbon drawing of the human Keap1 protein, a redox-regulated substrate adaptor for protein ubiquitination. Courtesy of Dr. Lesa Beamer, MU Dept. of Biochemistry. Published in *Journal of Biological Chemistry*, December 24, 2004, Vol. 279:54750-54758.

"The collaborative spirit of the science community at MU and the interdisciplinary approach to scientific investigation provided me an excellent training environment with great research opportunities . . . I felt ready and well-prepared for the new challenges I've encountered in my postdoctoral endeavors because of my experiences at MU."

Latisha Love-Gregory, PhD
Dr. Love-Gregory graduated from MU with a PhD in Genetics and is now a clinical fellow in chemistry and molecular diagnostics at Washington University in St. Louis, Mo.

Doctoral and Postdoctoral Fellowships and Degree Programs

Doctoral degrees are offered in over 40 life sciences departments and programs at MU. A variety of fellowships are available which include a competitive stipend, tuition waiver and health insurance. Graduate students in life science departments may also be eligible to participate in interdisciplinary NIH training grants. Postdoctoral fellowships are available in many departments and programs as well.

A sampling of life sciences programs can be found below. A complete listing of academic programs and campus information can be found at **http://www.missouri.edu**.

Biochemistry
http://www.biochem.missouri.edu
Phone toll free: (800) 647-2414

Biological Sciences
http://www.biology.missouri.edu
Phone toll free: (800) 553-5698

Biomedical and Health Informatics Research Training Fellowships
http://www.hmi.missouri.edu
Phone: (800) 877-4764

Comparative Medicine Program
http://www.radil.missouri.edu/info/cmp
Phone: (573) 882-6623

Conservation Biology
http://www.conserv.missouri.edu
Phone toll free: (800) 553-5698

Genetics Area Program
http://www.biotech.missouri.edu/genetics
Phone toll free: (877) Gene-PhD (436-3743)

Interdisciplinary Neuroscience Program
http://www.neurosci.missouri.edu
Phone toll free: (800) 553-5698

Interdisciplinary Plant Group
http://www.plantgroup.org
Phone: (573) 882-4847

Life Sciences Fellowships
http://lifesciences.missouri.edu
Phone toll free: (877) 436-3743

Medical Pharmacology & Physiology
http://www.missouri.edu/~maldz2/pharm/index.htm • **Phone:** (573) 882-4957

Mentored Biomedical Researchers Training Initiative
http://web.missouri.edu/~gradschl/financial/mbrti/mbrti.htm
Phone: (573) 884-2082

Molecular Microbiology & Immunology
http://www.missouri.edu/~mmiwww
Phone: (573) 882-8152

Pathology & Anatomical Sciences
Web: http://www.muhealth.org/~pathology
Phone: (573) 882-1201

Plant, Insect & Microbial Sciences
http://www.plantsci.missouri.edu
Phone: (573) 882-3001

Radiopharmaceutical Sciences Institute
http://www.muhealth.org/~rsi
Phone: (573) 882-2557

Veterinary Pathobiology
http://www.cvm.missouri.edu/vpbio
Phone: (573) 882-5034

an equal opportunity/ADA institution

To request ADA accommodations, call (573) 882-4544 or send e-mail to [illegible]@missouri.edu.

diversity

Broadening the Breadth of Science


GIBOR BASRI

In some cases, diversity appears to be improving. Greg Dewey—vice president for academic affairs, dean of faculty, and Finnigan Professor at the Keck Graduate Institute—says, "I would say that the most improvement over the last 10 years is in gender diversity. In the biological sciences, we are seeing much stronger participation by women, and that's creeping into chemistry. Math, physics, and engineering are not as diverse as you would want them to be. But we are seeing more women in biomedical engineering." He adds, though, "At the Ph.D. level, you are getting more gender diversity, but you're still not seeing a lot of women in higher rank academic positions."

Dewey also says, "Racial diversity is still a problem." He points out that academic science depends on a multicultural society. "Yet, you still have a problem with racial diversity in America, and that will be an ongoing problem because the pipeline of candidates is far from full."

For most young people, career aspirations often depend on role models. Scientists need that, too. "The main issue is getting people to become faculty members," says Basri, "and they might not enter the field if they don't see a lot of role models." He adds, "Young faculty members look to see if an institution looks like a congenial place to work, and they need to see someone they readily identify with there." Dewey agrees, saying, "Young faculty members need role models and mentors. If you don't have those role models in senior roles, that is a real problem."

Increasing Variety

In some medical fields, however, more diversity appears. Harry Selker, executive director of the Institute for Clinical Research and Health Policy Studies at Tufts-New England Medical Center, runs a cross-disciplinary, clinical research program. He says, "In clinical research, there are more members of the minorities and women in general internal medical research and health services than in subspecialty-oriented and bench-oriented research." He adds, "Traditionally, health services research has attracted people with social concerns that they wanted to see addressed, which may also explain the greater diversity."

Selker works in an extremely varied intellectual environment. His institute includes economists, political scientists, and sociologists as well as traditional clinical investigators, statisticians, and informatics experts. He says, "We feel strongly that there is a crucial advantage to an institute and a particular lab when it has a diversity of disciplines, from social to biological sciences." Still, that disciplinary diversity fails to meet all of Selker's criteria for a balanced environment. He also looks for what he calls personal diversity. "Different kinds of people—introverts and extroverts, detail-oriented and big-picture people, for examples—have different strengths," Selker says. "This turns out to be important because it provides a span of perspectives and thinking styles. We've benefited from that."


GREG DEWEY

Other scientists also believe that diversity extends beyond who scientists are to what they do. Craig Shimasaki, president and chief executive officer at InterGenetics, says, "A fully integrated diversity of disciplines is absolutely critical to science, but it is not yet fully embraced." He goes on to say, "People tend to fall back on what they are familiar with rather than expanding or broadening an approach to a problem. Instead, they just go deeper into what they've already done before."

Some studies—including the research on the predisposition to breast cancer being done at InterGenetics—demand an integrated team of scientists with a wide variety of skills. Shimasaki says, "We bring together geneticists, statisticians, and mathematicians with our molecular biologists." This is necessary, since approximately 90 percent of the women who contract breast cancer do not have a strong family history of the disease. "To tackle this disease," Shimasaki says, "you need to know the risk carriers." Right now, Shimasaki and his colleagues believe that this requires a cross-functional combination of biological and informatic sciences—a diversity of disciplines.

Instead of just being a numbers game, though, diversity can be an industrial culture. Jill Mueller, group vice president of human resources for the global pharmaceutical products group at Abbott, says, "We greatly value diversity of all kinds, including race, gender, and disabilities. It is absolutely key to our business and has been for as long as I can remember." She adds, "Just the other day, we were laughing that our campus looks like the United Nations because of the diversity of our people."

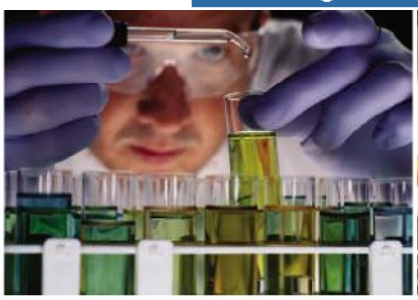
Enabling Disabilities

"A great many people who talk about diversity do not think it includes disabilities," says Virginia Stern, director of the AAAS Project on Science, Technology, & Disability and director of ENTRY POINT! The AAAS Project on Science, Technology, & Disability will celebrate its 30th anniversary at the association's annual meeting in February 2006. Stern says, "All these years, we've worked to bring role models to the forefront, but there is a shortage of role models with disabilities." To help create more role models in the future, Stern and her colleagues provide technical assistance to students, employers, families, and counselors. This project also publishes the *Resource Directory of Scientists and Engineers with Disabilities*, and the fourth addition is due out soon. Stern says, "This is the best and only source of role models."

In addition, ENTRY POINT! seeks out talented students with disabilities and arranges paid internships. "We have come to believe that these internships are critical in the pipeline for the companies," says Stern, "because they have a chance to know students with disabilities, working with them over the summer. It is really an CONTINUED »

scios

Focusing Science to ADVANCE MEDICINE


Scios Inc., headquartered in Fremont, California, a member of the Johnson & Johnson family of companies, is changing the way heart failure is treated. We are developing new and exciting therapeutics for cardiovascular disease, inflammatory disease and cancer. Our winning combination of integrated research, clinical experience, and passionate commitment to patients with unmet medical needs is making a difference in the management of disease. Innovation, collaboration and education are the cornerstones of Scios' culture.

We provide mentoring and career development opportunities throughout our organization. Our ability to grow as a company depends on finding and nurturing individuals with entrepreneurial spirit and passion to change the way patients are treated.

To support our continued growth, we seek candidates in the following areas:

CLINICAL RESEARCH

- Clinical Data Coder
- Clinical Research Associates
- Directors
- Lead - Clinical Data Mangement
- Manager, Supervisor - Clinical Supplies
- Managers
- Scientist

RESEARCH

- Associate Director, Bioanalytics
- Post Doc - Cell Biology
- Managers - Bioanalytics
- Medical Writers
- Research Associates, Formulations, HTS, Analytical Chemistry
- Scientists - Genomics, Informatics Toxicology, Analytical Chemistry, Pharmacokinetics

MEDICAL AFFAIRS

- Associate Director
- Directors
- Managers
- Medical Education
- Medical Writer
- Manager, Medical Education
- Senior Biostatistician, Lead, Associate Director - Biostatistics

REGULATORY AFFAIRS

- Associates
- Directors
- Managers

QUALITY CONTROL/ QUALITY ASSURANCE

- Analysts
- Specialist

For more information about us, to view a complete job description, and to apply online, please visit us at:

www.sciosinc.com

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Johnson & Johnson

diversity

Broadening the Breadth of Science


HARRY SELKER

entry point into the professions." This program receives private support from IBM and Merck, plus public support from NASA and the National Oceanic & Atmospheric Administration.

Today, the impact of decades of advocacy for people with disabilities can be seen in many institutions. At Tufts-New England Medical Center, Selker says, "One member of our research group was hit by a falling tree and became paralyzed and wheel chair bound." To help this scientist, Selker and his colleagues secured a grant supplement to her NIH grant and created a new entrance ramp to the building and changed doorways on her floor—all to make the facility more accessible. "It's all about treating people the way you want to be treated," says Selker. He adds, "NIH provided the supplement, and it was relatively easy to get it funded. NIH was a great partner in this."

Other universities also work to make life easier for scientists with disabilities. At Berkeley, Basri says, "My department right now has a quadriplegic student." He adds, "Berkeley in general is especially friendly toward people with disabilities. There is lots of help for them, and it is a pretty friendly place for that." Basri also mentions that people with limited mobility can focus on computer based research.

Removing the Obstacles

To make today's world of engineering and science even more friendly to people with disabilities, Stern says, "You need a champion, someone who knows that diversity includes disability." She explains that a manager in a company or agency who had a positive experience with a disabled scientist, engineer, or student becomes a champion. She adds, "Internships make it so managers and mentors gain experience that someone with a disability can be productive, creative, a team player, and an outstanding problem solver. In fact, anyone with a disability from birth or an accident develops persistence, which is basic to science and provides the ability to think out of the box."

Many of the obstacles for a person with a disability start long before reaching a professional career. So Stern and her colleagues reach out to students all the way down to preschool. She says, "We show them that science and engineering are viable careers. If counselors don't think science and engineering are possible careers then they are not going to encourage students with disabilities to get in college prep math courses. Then, when these students get to college, some doors are already closing. It takes an extra effort." Sometimes that effort includes assistive technology.

Disabilities can also affect scientists later in their careers, just from aging effects. Stern says, "I get calls several times a year from AAAS members who are losing their vision." She adds, "Sometimes a spouse or partner calls and realizes that the scientist is getting increasingly frustrated because he or she cannot read anymore or cannot read comfortably." In those cases, Stern and her colleagues put the person in touch with experts who can help.


VIRGINIA STERN

Spreading Diversity

Understanding the state of diversity can be easier than improving it. Moreover, the problems stretch from the past and into the future. Basri says, "The real problem is in grades K-12, and scientific leaders don't have much control over that. Still, we can work with K-12 leaders to see what we can do." He adds, "The state of science education in this country affects the under-represented populations more than others."

Still, Basri sees things that can be done. "Leaders can point out when they have found something that works, and share it with other leaders." Nonetheless, he adds that search committees must pick from small pools, in terms of underrepresented populations.

Dewey of the Keck Graduate Institute also sees potential approaches to improving diversity. He says, "Top scientists are incredibly influential, probably more so than they realize. They can be champions for young people and really help their careers." In addition, the Keck Graduate Institute also makes life easier for young faculty members by using a contract system, instead of tenure. For example, Dewey says, "We had a woman who came to us as an assistant professor, and she had a child in the year of her arrival. She negotiated that upfront, but she even said that she never would have done that in a tenure system."

At Abbott, Mueller sees many things that employees do to improve the company's diversity. She says, "We have several forums and networks that are employee-run and sponsored by executives. They include the Black Business Network, Chinese Culture Network, Women Leaders in Action, and others." She adds that these groups can make a large company seem like a smaller place that employees can navigate. "It is very important for scientists to meet colleagues in functions outside of R&D, as well as in the lab," she says.

Following the Results

Although one of the first steps to increasing diversity involves improving the breadth of backgrounds in an organization, other steps remain. Basri at Berkeley says, "Once you get more diversity in a department or institution, it is essential that you pay attention to whether those people are flourishing." So keeping track of a diverse staff makes the difference between success and failure.

In the end, making sure that a staff is diverse and productive enhances any organization. "The importance of innovative science is key in any industry," says Mueller. "That's how we run our business, because that diverse experience from different areas and countries is critical to moving innovative science forward."

Mike May (mikemay@mindspring.com) is a publishing consultant for science and technology based in Madison, Indiana, U.S.A.

The University of Georgia

New Faculty Positions in Biomedical Sciences

The University of Georgia is dramatically expanding its research programs in interdisciplinary biomedical and health sciences and is recruiting tenure track or tenured faculty in a number of related areas. This exciting initiative is marked by expansion of the Biomedical Health Sciences Institute, the opening of the Paul D. Coverdell Center for Interdisciplinary Biomedical Studies, the establishment of a new College of Public Health, the development of new, state-of-the-art bio-containment facilities for studies of animal and human health, and increasing emphasis on quantitative approaches to biological and medical problems. The University seeks to expand existing strengths by recruiting faculty in the following areas:

- **Genetics of complex traits relevant to human health and disease**
- **Pathogenesis, bioinformatics or host cellular response to emerging and re- emerging bacterial infectious diseases**
- **Immunology**
- **Molecular epidemiology**
- **Public Health: biostatistics, epidemiology, and health policy**
- **Systems biology, especially combined use of experimental and computational approaches to studies of signaling in complex contexts, such as cancer**
- **Glycoscience, especially glycosaminoglycan structure/function, glyco-immunology, cell/developmental, cancer or chemical biology**
- **Drug discovery**

Successful candidates will have the opportunity to participate in a rich interdisciplinary and collegial environment provided by the center and institute structure at UGA (described at **http://www.uga.edu/research/centers1.html**), and to benefit from partnerships involving nearby institutions, including the Centers for Disease Control and Prevention, the Medical College of Georgia, Georgia Tech and Emory University.

Applicants for any of these positions must have an advanced degree (PhD, DVM, MD or equivalent). Successful candidates will be expected to establish nationally recognized, externally funded research programs and contribute to teaching in undergraduate, graduate and professional training programs.

For more information regarding these positions, and for application instructions, please visit: **http://www.ovpr.uga.edu/facultypositions/**.

The University of Georgia is an Affirmative Action and Equal Opportunity Employer.

BIOCHEMISTRY FACULTY POSITION

Albert Einstein College of Medicine

The Dept of Biochemistry at the Albert Einstein College of Medicine, Yeshiva University, is seeking a tenure-track Assistant Professor. Applicants should demonstrate novel and innovative approaches in research. Research programs with focus on fundamental questions of biological chemistry with links to human disease are desirable.

Our faculty has exceptional and expanding research programs in structural biochemistry, systems and computational biology, chemical biology, membrane proteins, metabolic regulation, dynamics in catalysis, target identification, catalytic mechanisms, protein-nucleic acid interactions, genetics, inhibitor design and antibiotic resistance mechanisms. Selected research programs will be expanded into a new center for genetic and translational medicine now under construction.

Candidates are expected to have a PhD or MD degree, postdoctoral experience and a strong publication record. Applicants should send a curriculum vitae and a summary of research plans as a single PDF file to: **BCsearch05@medusa.bioc.aecom.yu.edu.** Letters from three or more references should be sent to the same address or to: **Search Committee, Dept of Biochemistry, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10461.** EOE

ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY
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SCOTT & WHITE

College of Medicine
The Texas A&M University System Health Science Center

Pediatric Hematology-Oncologist

The Section of Pediatric Hematology/Oncology at **Scott and White Clinic** and the **Texas A&M University System Health Science Center College of Medicine** (TAMUS HSC-COM) are seeking a clinician scientist with current research grants for a faculty position in a rapidly growing program. The candidate should be BE/BC in pediatric oncology and committed to an academic career. The successful candidates will join and enhance ongoing efforts in basic and translational research, with an institutional commitment to building a world-class experimental therapeutics program. An outstanding start-up package includes high quality laboratory space, excellent benefits and competitive salaries commensurate with academic qualifications. The position guarantees 75% protected time for research activities.

Scott & White Clinic is a 500+ physician directed multi-specialty group practice that is the leading provider of cancer care in Central Texas. Scott and White Clinic and the 486 bed tertiary Scott & White Memorial Hospital is the main clinical teaching facility for TAMUS HSC-COM. Outstanding clinical practice and laboratory facilities on campus that perform state of the art molecular and cellular biology research, flow cytometry, genomics and biostatistics are in place to support the research effort.

Please contact: **Don Wilson, M.D. Professor and Chairman, Department of Pediatrics, Scott & White, 2401 S. 31st, Temple, TX 76508. (800)725-3627 dwilson@swmail.sw.org Fax (254) 724-4974.**

For more information about Scott & White, please visit www.sw.org For Texas A&M www.tamhsc.edu. Scott & White is an equal opportunity employer.

Instructor or Assistant Professor Faculty Position
Building Interdisciplinary Research Careers in Women's Health (BIRCWH) Scholar

Brigham and Women's Hospital, Boston, MA is the home site for a Harvard-wide training grant initiative called "Hormones and Genes in Women's Health: From Bench to Bedside." We are pleased to invite applications for a full-time or part-time faculty position for an MD or PhD/ScD to develop an academic career in women's health. Specifically, this NIH-sponsored training grant will support scholars involved in investigating the role of hormones and genes in understanding sex differences in vulnerabilities to clinical disorders and those disorders specific to women. Potential Scholars investigating socio-environmental factors modulating the impact of hormones and genes in understanding these sex-specific vulnerabilities will also be considered. Scholars will be sponsored for up to five years of mentored research from Harvard faculty in basic, translational, or clinical aspects of women's health, and will combine, if applicable, clinical work in the Scholar's specialty. Successful applicants will have prior relevant research training in research methodology, potential to obtain independent research funding and evidence of publication in peer-reviewed journals. Faculty appointments will be at the Instructor or Assistant Professor level, commensurate with qualifications.

Applications are accepted until **January 10, 2006**. To apply, please email or mail CV and a 2-3 page summary on background, research interests and goals to: **Dr. Ursula Kaiser, Program Director, at BIRCWH@partners.org, Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115.**

The Brigham and Women's Hospital, an Affirmative Action, Equal Opportunity Employer, is a Harvard Medical School affiliate. Applications from women and minorities are highly encouraged and desired. Applicants must be US citizens or permanent residents.

CHAIR
Department of Medical Pharmacology and Toxicology

The University of California, Davis School of Medicine is seeking candidates for the Chair of the Department of Pharmacology and Toxicology. We seek an outstanding scientist with a superb record in research relevant to academic pharmacology to provide visionary and dynamic leadership to a vibrant and young department. The department is housed in the newly opened Genome and Biomedical Science Facility on the Davis campus, and has strong links to the new UC Davis Genome Center. The candidate should have a strong vision for basic science, and be prepared to lead the department in the School of Medicine's multi-departmental quest for excellence. The candidate should have demonstrated ability to meet the challenges of academic medicine and to work cooperatively and collegially within a diverse environment.

The Chair will lead a department that currently has 10 full-time faculty, nine of whom have joined the department within the last two years. Additional information about the department is available at: **http://som.ucdavis.edu/departments/pharmacology/**. The Chair will also be responsible for continued growth of the Department, with the addition of 3 new state-funded tenure track faculty positions. Each new position will be accompanied by the resources needed to ensure recruitment of outstanding faculty.

The successful candidate will be an internationally recognized scientist with an active research program who has a demonstrated record of leadership in research, education, mentoring, and administration, and who qualifies for appointment at the Full Professor level. The candidate must possess a Ph.D., M.D., M.D./Ph.D. or equivalent. This is a state funded position (FTE) within the School of Medicine.

Please forward: (1) curriculum vitae; (2) statement of research and administrative background; and (3) names and addresses of five references to: Pharmacology Chair Search Committee, via email to **Janice.weir@ucdmc.ucdavis.edu**, or via regular mail to: **Janice Weir, c/o Office of Academic Affairs, School of Medicine, University of California, Davis, Medical Center, PSSB Suite 2500, 4150 V Street, Sacramento, CA 95817**. For full consideration, applications must be received by **January 31, 2006**. The position will remain open until filled through June 30, 2006.

The University of California is an Affirmative Action/ Equal Opportunity Employer.

Associate Laboratory Animal Veterinarian
Division of Comparative Medicine
New England Primate Research Center
Harvard Medical School

The New England Primate Research Center (NEPRC) of Harvard Medical School has an immediate opening for a Laboratory Animal Veterinarian in the Division of Comparative Medicine. The academic appointment will be at the Research Associate/Instructor level in the Department of Pathology at Harvard Medical School. Responsibilities will include providing clinical care and veterinary support for a colony of more than 1,800 animals representing 9 species of Old and New World primates.

The NEPRC fosters a highly interactive and multidisciplinary research environment, and faculty in the Division of Comparative Medicine collaborate extensively with core scientists as well as investigators throughout the New England region. Faculty have long standing and well funded programs focused on behavior, neuroscience and infectious disease research. The veterinary staff is highly encouraged to pursue such independent or collaborative research. The Division of Comparative Medicine has excellent infrastructure to support both clinical and experimental medicine including recently completed and state-of-the-art laboratory, veterinary and biocontainment facilities. Minimum qualifications include a DVM (or equivalent). Previous experience with nonhuman primates is desirable and individuals with independent research experience are encouraged to apply.

The NEPRC is located in rural Southborough, MA, approximately 25 miles west of Boston, and in the heart of New England. Salary will be competitive and commensurate with experience. Additional information may be obtained at our website: **http://www.hms.harvard.edu/nerprc/**. To apply, send a letter indicating interests and experience, a curriculum vitae, and the names of three individuals who may be contacted for references to: **Keith Mansfield, DVM, Division of Comparative Medicine, New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, P.O. Box 9102, Southborough, MA 01772-9102.**

Harvard University is an Affirmative Action and Equal Opportunity Educator and Employer. Women and individuals from under-represented minorities are strongly encouraged to apply.

ALBERT-LUDWIGS-
UNIVERSITÄT FREIBURG

The Faculty of Chemistry, Pharmacy and Earth Sciences at the University of Freiburg invites applications for a

Full Professorship in Physical Chemistry (German W3 level, successor to Prof. G. Kothe).

The position is available from October 2006. Candidates are expected to teach physical chemistry at all levels and to represent the field scientifically. We seek a molecular spectroscopist with a high international reputation. Due to the faculty's focus on materials and life sciences, preference will be given to candidates that can contribute to these fields.

In the case of a first appointment to a university lectureship, the position is initially limited to a time interval of five years, a prolongation is subject to a positive evaluation. This limitation can be relaxed for candidates from abroad or from outside a university.

Freiburg University is an equal opportunities employer and particularly invites applications by female and handicapped scientists, which will be given preference provided they have a corresponding qualification.

Applications must be received at the following address by January 5th 2006: Dekan der Fakultät für Chemie, Pharmazie und Geowissenschaften, Universität Freiburg, Hebelstr. 27, D-79104 Freiburg im Breisgau, Germany.

Faculty Positions
UNMC Eppley Institute for Research in Cancer and Allied Diseases

The **Eppley Institute for Research in Cancer and Allied Diseases**, a multi-disciplinary cancer research institute at the **University of Nebraska Medical Center (UNMC)**, invites applications for tenure-leading positions at all levels. We seek candidates with outstanding record of research achievement with interests relevant to cancer research including, but not limited to: control of cell growth and death, regulation of gene expression, oncogene and tumor suppressor function, tumor immunology, animal models, metastasis and angiogenesis, chemical biology, cancer etiology and chemoprevention. We encourage applications from researchers focusing on basic molecular and cellular mechanisms, as well as those focusing on molecular therapeutics and specific disease models.

The Eppley Institute for Research in Cancer and Allied Diseases, an integral part of both the University of Nebraska Medical Center and the UNMC Cancer Center (NCI-designated Cancer Center), continues aggressive recruitment of outstanding scientists in several areas of scientific priority. The Institute provides a supportive environment that fosters creative, multidisciplinary research with world-class laboratory facilities, state of the art core facilities, and outstanding institutional and state support. New faculty will find a collaborative scientific environment coupled with very competitive start-up packages. Both pre- and post-doctoral fellowships are available for support of trainees. Omaha, the nation's 42nd largest city, offers an outstanding school system, low cost of living, and numerous recreational activities.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Applicants can apply online to position # 0831 at **https://jobs.unmc.edu**. Additional information can be found at **http://www.unmc.edu/cancercenter/**. Candidates should also forward a minimum of 3 letters of reference to: **Search Committee, Eppley Institute for Research in Cancer and Allied Diseases, Attn: Matt Winfrey, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, Nebraska, 68198-6805.**

The University of Nebraska Medical Center is an Equal Opportunity Employer.

UNCF•MERCK SCIENCE INITIATIVE

"A mind is a terrible thing to waste"

MERCK

UNDERGRADUATE SCIENCE RESEARCH SCHOLARSHIP AWARDS

- 15 Awards Annually
- Scholarships up to $25,000
- Two Summer Internships at a Merck Research Facility

An applicant must:

- Be a full-time student at any four-year college or university
- Have junior year academic status
- Major in a life or physical science (first professional degrees excluded)
- Have a minimum cumulative GPA of 3.3 (4.0 point scale)

GRADUATE SCIENCE RESEARCH DISSERTATION FELLOWSHIPS

- 12 Fellowships Annually
- Fellowship Stipends up to $42,000
- Department Grants of $10,000
- Support for 12-24 months

An applicant must:

- Be enrolled full-time in a Ph.D. or equivalent doctoral program in a biomedical life or physical science
- Be engaged in and within 1-3 years of completing dissertation research

POSTDOCTORAL SCIENCE RESEARCH FELLOWSHIPS

- 10 Fellowships Annually
- Fellowship Stipends up to $70,000
- Department Grants of $15,000
- Support for 12-24 months

An applicant must:

- Hold a Ph.D. or equivalent degree in a biomedical life or physical science
- Be appointed as a new or continuing postdoctoral fellow by the end of 2006 at an academic or non-academic research institution (private industrial laboratories are excluded)

Applicants must be African American (Black), U.S. citizens or permanent residents, and attending an institution in the U.S.A. Applications must be postmarked by December 15, 2005
For application forms and more information, please contact your department chairperson or Jerry L. Bryant, Ph.D., at the **United Negro College Fund**, 8260 Willow Oaks Corporate Drive, P.O. Box 10444, Fairfax, VA 22031-4511, by fax (703) 205-3574, by e-mail at ***uncfmerck@uncf.org***. Apply online or download from our website at ***www.uncf.org/merck/***

Tenure-Track Faculty Position, Microbial Pathogenesis, Yale School of Medicine

The Section of Microbial Pathogenesis of the Yale School of Medicine is seeking applicants for a tenure-track faculty position at the Assistant Professor level. Applications at other ranks from more established investigators with a strong record of accomplishments will also be considered. We are seeking applicants using multidisciplinary approaches to investigate host pathogen interactions. Individuals studying viral, bacterial, or protozoan organisms who are interested in the cell biology or immunobiology of host infection are encouraged to apply. The position offers an attractive start-up package, excellent laboratory space and a stimulating scientific research environment. Candidates should have a Ph.D. and/or M. D. degrees, suitable postdoctoral research experience, a strong record of research accomplishments, a commitment to develop independent, innovative research programs, and an interest in graduate and medical education.

Applicants should submit, a curriculum vitae; a statement of current and future research interests and arrange to have three letters of reference sent to: **Chair, Search Committee, Section of Microbial Pathogenesis, Yale School of Medicine, Boyer Center for Molecular Medicine, 295 Congress Av. New Haven, CT 06536.**

GRADUATE PROGRAM

University of Nebraska Medical Center

Graduate Studies in the Life Sciences

NEBRASKA'S HEALTH SCIENCE CENTER

Graduate programs in the life sciences are offered at the University of Nebraska Medical Center (UNMC) in Omaha, Nebraska. Studies leading to the Ph.D. are available in eight basic science programs and one integrated, interdepartmental training program (MSIA). In addition, the BRTP may be used as a common entry path for most of the basic science programs. Numerous training and research grants as well as significant internal funding sources support students in these degree programs. In the 2005-2006 academic year, most full-time Ph.D. students are being supported by a stipend of $21,000 or more with remission of all tuition. Most students begin their research rotations and orientation program in July or mid-August.

The Ph.D. life science programs currently available at UNMC include:

Biomedical Research Training Program (BRTP; common entry program)
Biochemistry and Molecular Biology
Cancer Research
Cellular and Integrative Physiology
Genetics, Cell Biology and Anatomy
Medical Sciences Interdepartmental Area (MSIA)
Pathology and Microbiology
Pharmaceutical Sciences
Pharmacology and Experimental Neuroscience
Toxicology

Interested students should visit UNMC at **http://app1.unmc.edu/gradstudies/**. Apply online!

UNMC has experienced a rapid growth in the past five years with new research buildings and laboratories added to support the increase in research activity. The campus is a modern, academic health center consisting of four professional colleges (Medicine, Dentistry, Nursing and Pharmacy), the Munroe-Meyer Institute, the Eppley Institute for Research in Cancer and Allied Diseases, and the Graduate Studies program. Our partner, the Nebraska Medical Center is the primary clinical teaching site for UNMC. Our location in metropolitan Omaha allows convenient travel connections and a modest cost of living.

Information regarding all programs, as well as an online application can be accessed through the website at **http://app1.unmc.edu/gradstudies/**. Questions about UNMC Graduate Programs may be addressed to: **David Crouse, PhD, Executive Associate Dean for Graduate Studies, 987810 Nebraska Medical Center, Omaha, NE 68198-7810; phone: 402-559-6531; facsimile: 402-559-7845; e-mail: UNMCGraduateStudies@unmc.edu.**

University of Nebraska Medical Center is an Equal Opportunity, Affirmative Action Employer. Minorities and Women are Encouraged to Apply.

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Postdoctoral Positions
National Institute of Child Health and Human Development

Epigenetic Regulation of Gene Expression
Karl Pfeifer, Ph.D., pfeifer k@mail.nih.gov
http://gpp.nih.gov/Researchers/Members/NICHD/KarlPfeifer.htm

Cell Regulation by Phosphoinositide Messengers
Tamas Balla, M.D., Ph.D., ballat@mail.nih.gov
http://dir2.nichd.nih.gov/nichd/ERRB/ERRBtext/molsigtrans_text.htm

Biology of RNA Metabolism in Eukaryotes
Richard Maraia, M.D., maraiar@mail.nih.gov
http://eclipse.nichd.nih.gov/nichd/Maraia/Maraialabpage.html

Nutritional and Behavioral Aspects of Pediatric and Adolescent Obesity
Jack A. Yanovski, M.D., Ph.D., jy15i@nih.gov
http://eclipse.nichd.nih.gov/nichd/deb/ugo/ugo.htm

Middle Childhood and Early Adolescence
Marc H. Bornstein, Ph.D., Marc H Bornstein@nih.gov
http://www.cfr.nichd.nih.gov

Molecular & Cell Biology of Genetic Disorders of Bone
Joan Marini, M.D., Ph.D., oidoc@helix.nih.gov
http://eclipse.nichd.nih.gov/nichd/annualreport/2004/bcmb/bcmb.htm

Neuregulins in Neuronal Plasticity and Schizophrenia
Andres Buonanno, Ph.D., buonanno@mail.nih.gov
http://gpp.nih.gov/Researchers/Members/NICHD/AndresBuonanno.htm

Retrovirus Models in Yeast
Henry Levin, Ph.D., henry_levin@nih.gov
http://eclipse.nichd.nih.gov/nichd/lgrd/sete/index.htm

Biology of Skeletal Growth
Jeffrey Baron, M.D., jeffrey.baron@nih.gov
http://ugd.nichd.nih.gov/

Endocrinology Fellow
Lynnette Nieman, M.D., NiemanL@nih.gov
http://www.training.nih.gov/onlineapps/trainingprograms/applications/CLITP_AdList.aspx?AdID=TP-56

Postdoctoral Opportunities
Neuron-Glia Interactions & Synaptic Plasticity
National Institute of Child Heath and Human Development

Two openings: (a) Neuron-glia communication in nervous system development & plasticity. (b) Electrophysiology of hippocampal LTP. Electrophysiologist must be experienced in hippocampal slice LTP for studies of intracellular signaling & transcriptional regulation in synaptic plasticity. Neuron/glial biologist with experience in myelination or perisynaptic glia will study activity-dependent communication between axons and myelinating glia, & glial involvement in synaptic plasticity. The laboratory utilizes DNA micro-arrays, 2-photon imaging, & molecular methods to study intracellular signaling & transcriptional regulation in activity-dependent nervous system development & plasticity. Ph.D. & < 5 years postdoctoral experience required. Send CV, 3 letters of reference:

R. Douglas Fields, Ph.D., 35 Lincoln Dr., 35/2A211, Bethesda, MD 20892-3713
fieldsd@mail.nih.gov / http://nsdps.nichd.nih.gov/

Health Scientist Administrator

The National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Department of Health & Human Services (DHHS) is seeking applicants for a Health Scientist Administrator position in the Center for Biotechnology and Innovation (CBI). The position is for a Director of the Applied and Translational Research Program. This program emphasizes interdisciplinary/multidisciplinary, highly innovative approach that combines engineering, physics, biology and clinical dental medicine for the resorption/regeneration of craniofacial structures (e.g., teeth, bone, salivary glands, periodontal and temporomandibular joint structures, etc.). Relevant areas include: studies regarding the design of bio-inspired new dental composite/ceramic materials through biomimetic principles, a systems approach to the design and development of new biocompatible/inductive materials that can stimulate cells and tissues to regenerate and/or materials that can become integrated into the body; use of stem cells and biomimetic approaches in regenerating soft and hard tissues/structures of the craniofacial region; computational methods for multiple scaffold designs that can promote stem cell assembly into multi-dimensional structures; design and development of integrated microfluidic platforms based on multiple separation and detection technologies on a single chip in order to obtain inexpensive, rapid detection methodologies for biological processes in health and disease; development of delivery vehicles (nanoparticles, artificial matrices) and development of micro-environments where cells can be precisely placed, manipulated and then analyzed in real time. The incumbent will direct, administer and evaluate a portfolio of extramural grants, contracts and cooperative agreements and will stimulate interest in and provide advice to the extramural community regarding the respective portfolio. In addition, the incumbent will participate in funding decisions, policy development, as well as implementation and coordination with other programs both within and outside of the NIDCR. The applicant is required to have a D.D.S., D.M.D., M.D., Ph.D. (or equivalent doctoral degree). The salary range for this position is $88,369 to $114,862 per annum, commensurate with experience. This position has knowledges, skills and abilities (KSAs) that must be addressed in order for applicants to be considered. The full vacancy announcement can be viewed at www.usajobs under NIDCR-05-9808. Applications will be accepted until November 21, 2005. Please submit materials to: Elan Ey, Branch I, Office of Human Resources, NIH, 6707 Democracy Blvd., Suite 400, Bethesda, MD 20892-5482 or by email: elan.ey@nih.gov. U.S. Citizenship is required.

National Institute of General Medical Sciences
National Institutes of Health
Department of Health and Human Services

The National Institute of General Medical Sciences (NIGMS) in Bethesda, MD is seeking applications from outstanding candidates for a Health Scientist Administrator (HSA) position in the Pharmacological and Physiological Sciences Branch within the Pharmacology, Physiology, and Biological Chemistry Division. The recruiting branch currently supports research and training into understanding the basis of traumatic and burn injury and the perioperative period, the molecular basis of action of anesthetics, the mechanisms of and genetics underlying the actions of therapeutic drugs, and the development of predictive preclinical toxicology approaches.

The individual hired will be responsible for applying his/her clinical and research expertise to manage and develop research and training grants in NIGMS' broad areas of basic studies in pharmacological and physiological sciences, and to foster the translation of results from fundamental research areas into clinical studies. The person should have experience gained in a medical research institution and understand how research is conducted with human subjects or patients in a clinical setting. A background in at least one of the following areas is preferred: trauma, injury and recovery, or clinical pharmacology, or immune system biology, or alternatively in a cross-cutting area such as studies of the role of inflammation in the disease process or of molecular/cellular signaling in these systems. Experience in modern methods of genome or proteome analysis would also be desirable.

Applicants must possess an MD and/or PhD plus scientific knowledge in the fields of pharmacology, physiology, immunology, systems biology, medicine, or related fields. Applicants must be familiar with both clinical and laboratory approaches in his/her own field(s) of expertise. Experience in the NIH peer review and grant award process would be beneficial. Salary will be commensurate with qualifications, may include a physician's comparability allowance, and will have a full package of benefits. Detailed vacancy announcements **NIGMS-05-100271** and **NIGMS-05-100881** with the qualifications and application procedures are available at the NIGMS web page at **http://www.nigms.nih.gov/about/job_vacancies.html**. Questions about application procedures may be directed to **Erin Bandak at 301-594-2324**. Applications must be received by **January 4, 2006.**

Health Research in a Changing World

Fighting Diseases and Improving Lives

Tenure-Track Investigator Position in Immunology and Related Fields
National Institute of Allergy and Infectious Diseases
National Institutes of Health (NIH)

The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR) is recruiting for a Tenure-Track Investigator in the Laboratory of Cellular and Molecular Immunology (LCMI). The NIAID is a major research component of the NIH and the Department of Health and Human Services (DHHS).

The Laboratory of Cellular and Molecular Immunology (LCMI) is seeking an M.D., Ph.D., D.V.M., or an equivalent degree for a tenure track position. Candidates with a strong record of creative, scientific accomplishments, and those with a novel, progressive approach to the discipline are particularly encouraged to apply.

The successful candidate will have a unique opportunity to establish an independent research program at the NIH main campus in Bethesda, Maryland. This facility houses one of the largest immunological research communities in the world, with access to flow cytometry, confocal microscopy, mass spectrometry and microarray production. This position will have committed resources for space, a technician and two postdoctoral fellows, as well as an allocated budget to cover service, supplies, animals and salaries.

Salary will be commensurate with research experience and accomplishments. A full Civil Service package of benefits is available, including retirement, health, life, long term insurance care and Thrift Savings Plan.

Address any questions about this position to Dr. Ron Schwartz at rs34r@nih.gov. To apply, candidates must submit: curriculum vitae and bibliography, and a 2-3 page description of a proposed research program and selected publications, preferably via email to Ms. Felicia Braunstein at braunsteinf@niaid.nih.gov. In addition, three letters of recommendation must be sent to Ms. Felicia Braunstein, Committee Manager, NIAID, NIH; Bldg. 10, Rm.4A30, MSC-1349; Bethesda, MD 20892-1349. All applications must be received by December 1, 2005. All applicants will be notified by e-mail or phone when their applications are received and then complete.

We invite you to explore our Institute and other job opportunities at http://healthresearch.niaid.nih.gov/science.

Please reference "Science" on your resume.

CHAIR

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

ARIZONA STATE UNIVERSITY

The Department of Chemistry and Biochemistry at Arizona State University invites applications and nominations for the position of Department Chair. Arizona State University (see **http://www.asu.edu**) is a dynamic, internationally recognized research university serving more than 60,000 students in the Phoenix metropolitan area, one of the fastest growing urban centers in the nation. Chemistry and Biochemistry at Arizona State University currently has about 40 faculty and is embracing a period of unprecedented growth. The department has benefited from a University-wide initiative to upgrade research space and has hired ten new faculty members in the last two years (see **http://www.chemistry.asu.edu/** for more detailed information). The Department has a historically strong record in interdisciplinary research, recognized by a series of nationally funded centers, and takes special pride in a collegial atmosphere bolstered by permeable boundaries between traditional programs and strong ties to related academic units including the Department of Physics, the School of Earth and Space Exploration, the School of Materials, the School of Life Sciences, and the BioDesign Institute.

The successful candidate is expected to provide visionary leadership, to oversee growth in the department faculty and research programs, to further develop the graduate and undergraduate programs, to establish ties between the department and other University or community-based research and education initiatives, and to maintain a productive research program. Candidates must have an earned doctorate in chemistry, biochemistry, or a closely related field, achieved national and international recognition for their scholarship appropriate to the rank of Professor, have a distinguished scholarly record, and be familiar with the federal budget process and the strategic goals of the funding agencies. Desirable qualifications include documented leadership, previous administrative experience in a doctoral-granting department, a history of external funding, experience with program development in research and education, evidence of strong communication and organizational skills, and evidence of commitment in working with and supporting a diverse student and faculty population.

The position is available beginning July 1, 2006, or as soon as possible thereafter. Salary and start-up will be competitive and commensurate with qualifications. Nominations for this position are being sought and will be most helpful if received by **December 9, 2005**. Review of applications will begin on **January 5, 2006**; if not filled, applications will be evaluated every two weeks thereafter until the search is closed. Applicants must submit electronically (in MS Word or PDF format) a cover letter and a current curriculum vitae to **Ms. Roxana Martin (roxana.martin@asu.edu)**. Inquiries and nominations should be directed to: **Robert Page, Director, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501**; or email: **robert.page@asu.edu**. A background check is required for employment.

ASU is an Affirmative Action/Equal Opportunity Employer.

University of Heidelberg

The **Department of Physics and Astronomy** of the Ruprecht-Karls-University of Heidelberg, Germany, invites applications for a

Professorship in Experimental Physics

We are looking for an outstanding experimental physicist active in the field of atomic and quantum physics as well as related subjects in fundamental research. The position is equivalent to a chair in experimental physics. The new professor is expected to demonstrate a commitment to teaching excellence in both the undergraduate and the post graduate level.

The position is permanent. Candidates who have not served as a University Professor will initially be appointed on a fixed-term contract according to § 50 Paragraph 1 LHG (University Law of the Federal State of Baden-Württemberg), after which tenure may be granted without the necessity of re-application. Exceptions may be made for international or extra-university applicants.

The University of Heidelberg seeks to increase the number of qualified women in teaching and research positions and strongly encourages applications of women. Handicapped persons with equivalent qualifications will be given preference.

Qualified candidates are invited to submit their application until **31.12.2005** with the usual documents to **Prof. Dr. K. Meier, Dean, Department of Physics and Astronomy, Albert-Ueberle-Str. 3-5, D-69120 Heidelberg, Germany.**

DEAN

School of Natural Sciences and Mathematics

CSUB seeks an individual with vision and energy to become Dean of NSM and collaborate with our distinguished faculty in promoting excellence in research and teaching. The Dean will lead NSM in groundbreaking for a Math/Computer Science building and inaugurating master's degrees. NSM offers B.S. degrees in biology, chemistry, computer science, and physics and B.S. and master's degrees in geology, mathematics, and nursing. Detailed description available at www.csub.edu/AcademicAffairs/FacEmp/NSM.pdf

Qualifications:

- Doctorate and record to merit appointment as professor in an NSM discipline
- Academic administrative experience
- Success in external funding and building partnerships
- A record of effective collaboration
- Commitment to diversity

Applications accepted until position is filled. Interviews planned for January 2006. Appointment expected to begin July 3, 2006. Completed applications must include a letter of application, CV, and contact information for at least three references. Submit applications to:

Search Committee, NSM Dean
c/o Office of the Provost, 59 ADM
California State University, Bakersfield
Bakersfield, CA 93311-1022

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GBF MHH

The GBF and the MHH invite applications for presentations at a

Symposium on Experimental and Clinical Infectious Disease Research – Challenges and Clinical Applications –

The German Research Centre of Biotechnology (GBF) in Braunschweig and the Hannover Medical School (MHH), intend to set up a joint "Centre for Experimental and Clinical Infectious Disease Research" near the campus of the Hannover Medical School. The aim of the research centre is to establish itself at the forefront of infectious disease research and become a driving force in the translation of basic research into the development of new vaccines and antiinfectives.

In order to identify suitable candidates as

Director of Experimental Infectious Disease Research

and

Director of Clinical Infectious Disease Research

a symposium will be organised for the 16th and 17th February, 2006. The symposium will allow candidates to present themselves to an international advisory board that will support the subsequent recruitment process. Both positions are permanent and will involve appointment to a full professorship (W3) at the Hannover Medical School.

Following this symposium, candidates will therefore undergo the standard recruitment procedure for full professors as defined by university legislation in Lower Saxony. This procedure involves a formal application, an interview before a selection committee, and confirmation by the government of Lower Saxony.

In addition, there are two openings for the position of

Head of an Infectious Disease Research Group

These positions will be at the W2 level (Associate or Junior Professor).

Furthermore, the Centre wishes to set up a junior research group and recruit a

Head of a Young Investigator Group of Infectious Disease Research

- The appropriate candidate should work in the general field of infection research and have a sound scientific track record.
- Experience and interest in interdisciplinary work and translational research are essential.
- Development of a rigorous research program at the interface of basic experimental infectious disease research and clinical infectiology is expected.
- In particular, candidates with expertise in the following areas are encouraged to apply: genetic susceptibility of infectious disease, molecular mycology, infection and cancer, persistent and chronic infections.

Additional information about the Centre of Experimental and Clinical Infectious Disease Research is available at: **www.gbf.de/translation**

Please indicate on your application the position you wish to apply for and include the usual documents (CV, publications), along with a brief description (max of 10 pages) of the research programme you plan to implement.

Application deadline: Jan 6th, 2006

Please send your application to either:

Prof. Dr. Dieter Bitter-Suermann, President
Hannover Medical School
Carl-Neuberg-Strasse 1
30625 Hannover, Germany

Prof. Dr. Rudi Balling, Scientific Director
German Research Centre of Biotechnology (GBF)
Mascheroder Weg 1
38124 Braunschweig, Germany

Additional information can be obtained from

Prof. Dr. D. Bitter-Suermann
bitter-suermann.dieter@mh-hannover.de,
++49 (0)511.532-6000

Prof. Dr. R. Balling
balling@gbf.de,
++49 (0)531.6181-500

HELMHOLTZ GEMEINSCHAFT

The Helmholtz Association is an affiliation of 15 German research centres whose main work focus lies in science and technology, medicine and biology.

Our 24 000 employees are known for their outstanding achievements in six fields of scientific research: earth and environment, the structure of matter, energy, transport and aerospace, health and key technologies.

In cooperation with the Hannover Medical School, the GBF in Braunschweig, Germany – our research institute for infectious diseases – is seeking suitable candidates for various positions:

St. Jude Children's Research Hospital
ALSAC - Danny Thomas, Founder
Finding cures. Saving children.

FACULTY POSITION IN HIGH THROUGHPUT SCREENING

The Department of Chemical Biology and Therapeutics at **St. Jude Children's Research Hospital** invites applications for a faculty position at the level of ASSOCIATE MEMBER or MEMBER. We are specifically seeking applicants currently leading established research programs in the development and execution of high throughput enzymatic and/or cellular screens for the discovery of novel small molecules.

The Department of Chemical Biology and Therapeutics is one of 15 academic departments at St. Jude Children's Research Hospital. The Department includes laboratories focusing on parallel, medicinal, and analytical chemistry as well as new technologies and disease biology. The institute facilitates translational research, and has outstanding shared laboratory and clinical resources that facilitate collaborations among a highly collegial group of scientists. Extensive opportunities exist for collaboration with both clinically based and basic research programs relevant to oncology and infectious disease.

Appointees will lead a strong program in a multidisciplinary, thematically integrated Department focused on the discovery and development of small molecules for perturbing cellular functions - particularly in systems relevant to pediatric oncology and infectious disease. Individuals will contribute to one or more existing and new programs at the institution, including the interdisciplinary research programs of Developmental Therapeutics for Solid Malignancies, Hematological Malignancies, Infection & Host Defense, Molecular Oncology, Neurobiology & Brain Tumor, Signal Transduction, Transplantation & Gene Therapy, Chemical Biology, or Cancer Prevention & Control.

St. Jude offers a very competitive package for this position, including a generous startup allowance with newly remodeled space and equipment; laboratory resources (as needed); and support positions. In addition, appointees have access to a range of institutional core facilities for protein and nucleic acid chemistry, microarray analysis, gene knockout and transgenic technologies, pharmacokinetics, and development of animal models.

Those interested in joining this multidisciplinary department should arrange to have their CV, a brief prospectus of research interests, and three letters of recommendation sent to:

R. Kip Guy, Ph.D., Chair
Department of Chemical Biology and Therapeutics
St. Jude Children's Research Hospital
322 North Lauderdale Street • Memphis, TN 38105

www.stjude.org

SJCRH is an Affirmative Action/Equal Opportunity Employer

Faculty Position in *C. elegans* Molecular Genetics

The Department of Genetics, Cell Biology and Development (GCD) at the University of Minnesota is conducting a search for an Assistant or Associate Professor using molecular genetic approaches in the nematode *C. elegans* to study fundamental biological mechanisms. The successful candidate will join a core group of active nematode researchers with diverse interests including developmental timing, sexual differentiation, intercellular communication, nervous system development, cell adhesion, and the cellular roles of myosins and the cytoskeleton. The *C. elegans* group has significant ties to an interdepartmental Developmental Biology Center (DBC) (**http://www.med.umn.edu/dbc/**). GCD faculty exploit a variety of genetic model organisms, including fungi, algae, flies, zebrafish and mice. GCD is also home to the International *C. elegans* Genetics Center.

The Department of GCD will provide a competitive salary and start up package, plus excellent laboratory space, with access to state-of-the-art core facilities. The candidate must have a Ph.D. or M.D. and at least two years of postdoctoral experience. Investigators studying any aspect of *C. elegans* biology will be considered, with use of cutting edge technology, including genomics approaches, to address a fundamental problem serving as an important selection criterion. Emphasis will also be placed on the potential for interaction with existing research programs in GCD (**http://www.ged.med.umn.edu/**) and the DBC. The person selected will be expected to develop or expand upon an independent, externally funded research program and participate in the teaching mission of the department. Applications will be reviewed, beginning **December 1, 2005**, and will be accepted until the position is filled.

Please send a CV, a brief statement of current and future research, and three letters of reference to: **GCD *C. elegans* Faculty Search, c/o Mary Muwahid (muwah001@umn.edu), University of Minnesota, Department of Genetics, Cell Biology and Development, 6-160 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455.**

The University of Minnesota is an Equal Opportunity Educator and Employer.

UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE
DEPARTMENT OF OTOLARYNGOLOGY

Director of Auditory Research

The Department of Otolaryngology at the University of Pittsburgh School of Medicine is expanding its research initiatives in the area of auditory science. Successful candidates possessing a Ph.D. or M.D. degree will be expected to have established leadership in auditory research as well as develop and lead a hearing research program in a collaborative environment, through targeted recruitments. Otolaryngology research faculty have the opportunity to participate in several graduate programs as well as teach medical students, residents and fellows. Appointment will be at the Associate Professor or Professor level in the tenure track commensurate with the qualifications of the candidate. Joint appointment in the Departments of Neurobiology or Neuroscience or Psychology and the Center for the Neuronal Basis of Cognition will be encouraged. We offer a highly competitive start-up package, academic salary, fringe benefits, and state-of-the-art facilities. The University of Pittsburgh provides an exciting, vibrant, and highly interdisciplinary scientific community.

Qualified candidates should send a letter of interest and comprehensive curriculum vitae to:

Jennifer R. Grandis, M.D.
Professor and Vice Chair for Research
Department of Otolaryngology
University of Pittsburgh School of Medicine
200 Lothrop Street, EEI Suite 500
Pittsburgh PA 15213
boozerd@upmc.edu

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Virginia Tech

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Dean, College of Science

Virginia Polytechnic Institute and State University, known as Virginia Tech, invites nominations or expressions of interest for the position of Dean, College of Science.

Virginia Tech is the senior land grant university in the Commonwealth of Virginia with 21,627 full-time undergraduates and 6,352 graduate students enrolled both on- and off-campus throughout the state. The university's strategic plan sets ambitious goals to increase Virginia Tech's stature as one of the nation's leading research universities.

About the College of Science:

The College of Science at Virginia Tech provides students with strong training in analytical skills and a comprehensive foundation in the scientific method. Outstanding faculty members conduct research and teach courses in eight disciplines leading to baccalaureate and advanced degrees: biological sciences, chemistry, economics, geosciences, mathematics, physics, psychology, and statistics. The college also offers academic advising and appropriate preparatory coursework for students interested in pre-medicine, pre-dentistry, pre-veterinary medicine, and scientific law.

In addition to traditional majors, the college offers a graduate degree in macromolecular science and engineering, programs in nano-scale science and technology, computational science, and infectious diseases, and supports research centers—in areas such as biomedical and public health sciences, macromolecules and interfaces, and critical technology and applied science — that encompass other colleges at the university. Allied disciplines emphasize the study of behavioral science as well as economic and strategic decision-making. The college is committed to providing research opportunities for interested students at all levels.

Position Responsibilities:

- Advancing the vision for the college within the university's and college's strategic plans;
- Providing entrepreneurial leadership for the growth and development of academic, research, and outreach programs in the sciences;
- Further enhancing the diversity of the faculty, staff, researchers, and student body;
- Creating a climate, organizational structure, and managerial leadership team that encourage all members of the college community to contribute positively and productively to departmental, college, and university goals;
- Developing and maintaining productive relationships with external constituencies—government agencies, corporate partners, alumni, donors, advisory board members, and others;
- Serving as a vital member of the university's overall leadership team.

Required Qualifications:

- Earned doctorate and a distinguished record of scholarly activity that would qualify for rank of professor in an academic department in the college;
- Demonstrated effective communication and interpersonal skills, ability to work effectively in collaboration with many constituencies;
- Experience in leading or managing a major research program;
- Demonstrated successful leadership in higher education.

Desired Qualifications:

- Appreciation of the mission of a land-grant university;
- Vision and ability to advance the research agenda of the college and university, with emphases on interdisciplinary and cross-college initiatives;
- Ability to recognize and take advantage of rapid changes in the forefront of science;
- Demonstrated effectiveness in planning, administration, and personnel and fiscal management;
- Record of accomplishment in recruitment and retention of outstanding faculty, including women and minority faculty, staff, and students;
- Successful experience, or demonstrated potential, in fund-raising, development activities, and collaboration with industry.

Candidates must complete an application on-line at **www.jobs.vt.edu**, posting **043179**. Attach to the on-line application a letter of interest that addresses the responsibilities and qualifications stated above, current curriculum vitae, and the names of three references. To be assured of full consideration, applications should be received by **December 1, 2005**. The position will be filled as soon after that date as possible. Please see **http://www.provost.vt.edu/Resources.html** for helpful information for prospective faculty considering employment at Virginia Tech.

Nominations may be sent to: **Dr. Mark McNamee, University Provost and Vice President for Academic Affairs, Virginia Tech, 210 Burruss Hall, Blacksburg, VA 24061, mmcnamee@vt.edu.**

Individuals with disabilities desiring accommodations in the application process should contact **Suzie Karlin** at **skarlin@vt.edu** or **540-231-2350**.

Virginia Tech has a strong commitment to the principle of diversity and, in that spirit, seeks a broad spectrum of candidates including women, minorities, and people with disabilities. Virginia Tech is the recipient of a National Science Foundation ADVANCE Institutional Transformation Award to increase the participation of women in academic science and engineering careers.

Hauptman-Woodward Medical Research Institute
Research Scientists
Computational Structural Biology

The Hauptman-Woodward Medical Research Institute (HWI) is a private, not-for-profit organization studying the structures and functions of macromolecules of biomedical interest. HWI is part of the Buffalo-Niagara Medical Campus, a world-class consortium of research, clinical, and educational institutions located in downtown Buffalo, NY, USA. HWI is growing and has just occupied a new state-of-the art building.

HWI has a strong history in innovative computational methods development. To complement and enhance our current research efforts, we seek to recruit an additional independent computational scientist. We are looking for a structural biologist interested in developing methodology or, alternatively, a researcher who will make use of structural information to study areas that might include: determination of protein folding rules, domain interactions, evolution of structure and function and prediction of structure, prediction and analysis of protein-protein and protein-ligand interaction, novel protein design, molecular modeling including pharmaceutical design, biophysical modeling, and bioinformatics-related disciplines.

This new HWI Research Scientist will be hired at the equivalent of the Assistant, Associate, or full Professor level based on his or her qualifications. HWI scientists also serve as faculty within the Department of Structural Biology at the State University of New York at Buffalo.

For detailed information about our current research programs and facilities, visit our web site, **http://www.hwi.buffalo.edu**. Interested applicants should submit a curriculum vitae and research plan, and they should arrange to have three letters of reference sent to the address below. Review of applications will commence immediately. To ensure full consideration, applications must be received by February 1, 2006.

George T. DeTitta, Ph.D., Hauptman-Woodward Medical Research Institute, 700 Ellicott St., Buffalo, NY 14203-1102
Email recruitment@hwi.buffalo.edu

The Hauptman-Woodward Institute is an Equal Opportunity Employer

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The **Department of Molecular Biology and Microbiology** at the **Medical School of Tufts University** invites applicants for a tenure-track faculty position in Virology at the rank of Assistant Professor. Outstanding applicants at more senior ranks will also be considered. The successful candidate is expected to have, or to develop a productive and nationally funded research program. Applicants with research programs in all areas of human/animal virology and especially in aspects of molecular virology, emerging viral infections, viral pathogenesis, viral immunology and viral oncology are encouraged to apply.

The Department is a highly interactive group of virologists and microbiologists, with research programs in the areas of molecular genetics, microbial pathogenesis, pathogen-host interaction, yeast genetics, and retroviruses. Faculty members of the Department also participate in graduate programs in Molecular Microbiology, Genetics, Biochemistry and Immunology. Applicants must have a Ph.D. or M.D. degree or equivalent and are expected to contribute to the teaching of medical, dental, and graduate students. Laboratory space will be in the recently opened Jaharis Family Center for Biomedical and Nutrition Sciences on the Boston Health Sciences campus.

Applicants should submit a curriculum vitae, statement of research interests and future plans, and names of three references to: **Dr. John Coffin, Chair, Virology Search Committee, Department of Molecular Biology and Microbiology, Tufts University, 136 Harrison Avenue, Boston, MA 02111**. Electronic submission of applications is preferred (e-mail: **lauralyn.smith@tufts.edu**). Review of applications will commence upon receipt and will continue until the position is filled.

*Tufts University is committed to Affirmative Action, Equal Opportunity and the diversity of its workforce. Information is available at **http://www.tufts.edu/sackler/**.*

Medical College of Georgia
Department of Physiology
Faculty Positions in
Cardiovascular and Renal Physiology

The Department of Physiology invites applications for three tenure-track positions. The rank of the appointment (Assistant Professor/Associate Professor/Professor) will be commensurate with the qualifications and experience of the successful candidate. A degree in medicine, veterinary medicine or Ph.D. in biological sciences with postdoctoral research experience is required. Successful candidates are expected to establish active independent programs of extramurally funded research to complement research strengths and goals of the department and the medical college. Our department focuses on questions of cellular signaling, neural regulation and hormonal control in a broad range of model systems of cardiovascular and renal disease. Successful candidates will receive substantial start-up packages and be housed in newly constructed/renovated facilities. There is a strong institutional commitment to core facilities, graduate programs and an interdisciplinary approach. Applicants are also expected to have teaching experience and be committed to teaching students in the schools of medicine, allied health sciences and graduate studies.

Applicants should submit a curriculum vitae, a statement of research interests and career goals and three letters of reference to:

Michael W. Brands, Ph.D.
Search Committee Chair
Department of Physiology
1120 Fifteenth Street
Medical College of Georgia
Augusta, Georgia 30912-3000

For full consideration, applications should be received by **December 31, 2005**.

The Medical College of Georgia is committed to diversity in attracting faculty to fill these positions and is an Affirmative Action/Equal Access Institution.

BCM
Baylor College of Medicine

TENURED/TENURE TRACK FACULTY POSITIONS IN GENETICS AND GENETIC INSTABILITY
Department of Molecular and Human Genetics

Baylor College of Medicine (BCM) seeks nominations and applications for tenured/tenure-track faculty positions at any level in the Department of Molecular and Human Genetics a world leader in genetic research. At least one position will be filled in the area of genetic/genomic instability (with possible secondary appointment to the BCM Cancer Center, **http://www.bcm.edu/cancercenter/**) and at least one position in any aspect of genetics. The department is composed of more than 40 primary faculty members whose research interests include genomics, mammalian development, the metabolic and genetic bases for inherited human disease, gene therapy, gene structure and expression, mechanisms of DNA replication and repair, mutation, DNA recombination, genetic instability and cancer, cytogenetics, behavioral genetics, bioinformatics, and the biology of aging. Departmental research includes strengths in human, bacterial, mouse, yeast, Drosophila and Dictyostelium genetics. The department currently ranks first, by a wide margin, in number of NIH grants and first in overall NIH funding in genetics departments at US medical schools. See **http://www.imgen.bcm.tmc.edu/molgen/**.

Successful candidates will have strong basic research programs, for one position in genetic/genomic instability, including but not limited to DNA repair, mutation, replication, genome rearrangement, DNA damage response, or cancer studied in any organism, and for another position in any aspect of genetics including mouse genetics or the genetics of any model organism. A search in human genetics will be posted separately. Generous start-up support is available.

Letters of nomination or curricula vitarum should be sent with a brief summary of research plans, and names, addresses, and phone numbers of at least three references to: **Susan M Rosenberg, Ph.D., Chair of Search Committee, Department of Molecular and Human Genetics, Baylor College of Medicine, MSC-BCM225, One Baylor Plaza, Houston, TX, 77030-3411; E-mail: smr@bcm.tmc.edu**.

Baylor College of Medicine is an Equal Opportunity, Affirmative Action and Equal Access Employer.

Vacancies for one Experienced Researcher & one Early-stage Researcher European Community – Marie Curie Excellence Team – Molecular & Cellular Biology of Primary Immune Deficiencies – Host Laboratory: INSERM U563, Toulouse, France – Starting date: February 2006

Two fellowships funded by the European Community are available to join a new Marie Curie Excellence Team at INSERM U563, Purpan University Hospital, Toulouse, France.

The major area of expertise of the Team Leader (L. DUPRE) is the characterization of molecular mechanisms that regulate human T lymphocyte activation (Immunity 2002,17:157; N. Engl. J. Med. 2004,351:1419 ; Blood 2005,105:4383). The objective of the Team is to study the molecular defects of cytotoxic T lymphocytes isolated from patients affected by life-threatening primary immune deficiencies. This project at the interface between fundamental and clinical research will be instrumental for the development of gene therapy approaches.

The Team benefits from a stimulating environment in the fields of T lymphocyte biology and clinical immunology and has established strong collaborative links.

The Experienced Researcher (PhD, 4-10 years post graduate experience) will be in charge of conducting biochemical experiments to identify proteins involved in the regulation of cytotoxic T lymphocyte activation and function. The precise role of selected proteins will be studied using gene transfer technology and live microscopy (already in place). A scientist with expertise in protein chemistry (mass spectrometry and bioinformatics) will be selected. Experience in molecular/cellular immunology will be a plus.

The Early-stage Researcher (0-4 years post graduate experience with motivation to enter a PhD program) will construct retroviral vectors encoding proteins involved in the regulation of cytotoxic T lymphocyte activation and function. The researcher will be in charge of the gene transfer experiments in lymphocytes isolated from patients with defective cytotoxic function. The candidate should have experience in molecular biology and cellular immunology.

PROFILE OF THE CANDIDATES:
Successful candidates will be enthusiastic, highly motivated and have a strong propensity to work in a transnational team. Fluency in English is required. No nationality restriction applies. Equal opportunity will be given for women and men.

CONTRACTS:
Four-year full-employment contracts including living, travel and mobility allowances. Competitive Salaries.

APPLICATIONS:
Please include CV, statement of interests & future goals, as well as names of two referees.

CONTACT & INFORMATION REQUESTS:
L. DUPRE, E-mail: l.dupre@hsr.it

Inserm
institut national de la santé et de la recherche médicale

GKSS FORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

CARL VON OSSIETZKY universität OLDENBURG

Unternehmen Großforschung

Grundlagen für morgen

The GKSS Research Centre in Geesthacht near Hamburg is a member of the Hermann von Helmholtz Association of National Research Centres and has a branch institute in Teltow near Berlin. Its 700 employees cooperate with various universities and industrial firms to conduct research and development work in the areas of coastal research, advanced engineering materials, regenerative medicine, and structure research with neutrons and synchrotron radiation.

In a joint appointment process with the University of Oldenburg, GKSS is seeking to fill the vacant position of **Director** of the Department of **Data Analysis and Data Assimilation** in the **System Analysis and Modelling** section **(Prof. von Storch)** of the **Institute for Coastal Research at GKSS.** The position is based in Geesthacht.

Associated with the position is a

Professorship (W2)

in the Faculty of Mathematics and Natural Sciences at the University of Oldenburg in the field of coastal research of the Institute for Chemistry and Biology of the Marine Environment (ICBM), with the obligation to teach subjects relevant to the ICBM for 2 hours per week during term.

One of the key areas of expertise at the GKSS Institute for Coastal Research is the development of methods for environmental monitoring and forecasting. In this field, one of GKSS' main strengths is the observation and modelling of currents and sea conditions. GKSS has been successfully cooperating with the ICBM for many years on the observation, modelling and analysis of physical, chemical and ecological processes in tidal flats. In this area, GKSS has a long history of developing and testing operational methods for remote observation and the creation of complex models.

At GKSS, the appointee is expected to contribute his/her expertise to the programme titled "Coastal Change: Long-term Trends and Extreme Events", and in particular to topics related to currents and sea conditions. A special focus here is the development of observation and modelling methods for operational use in coastal waters. The appointee will conduct research that is closely linked to the ICBM programme in Oldenburg as well as to the Marine, Coastal and Polar Research programme in Geesthacht.

Applicants are required to have a university degree in mathematics, physics or another discipline suited to the professorship in question. They should also have earned a doctorate and a postdoctoral degree or possess comparable academic qualifications.

The University of Oldenburg and GKSS wish to increase the number of female scientists in leadership positions. Female researchers are therefore particularly encouraged to apply. Preference will be given to severely disabled applicants with the appropriate qualifications.

For additional information, please contact Prof. Hans von Storch (storch@gkss.de) or Prof. Jörg-Olaf Wolff (wolff@icbm.de).

The deadline for applications is 23. December 2005. Please submit written applications along with the usual documents (including a description of your scientific career and teaching experience, a list of publications and copies of your three most important publications) to:

Carl-von-Ossietzky Universität Oldenburg
Dekanat Fakultät V
Postfach 2053 • 26111 Oldenburg • Germany

POSITIONS OPEN

BIOLOGY FACULTY POSITIONS

The Department of Biological Sciences at Wayne State University has three tenure track openings for new Faculty. Rank will be dependent upon qualifications. Preference will be given to candidates who utilize state of the art approaches to study complex biological problems with the potential to integrate their research programs with existing multidisciplinary research groups.

Ecology/organismal biology. Areas of interest include, but are not limited to, aquatic ecology, behavioral ecology, evolutionary ecology, microbial ecology, phylobiogeography, organismal biology and population ecology. Position Number 032356.

Microbiology. Areas of interest include, but are not limited to, molecular microbiology, medical microbiology, microbial genomics, bioinformatics, microbial physiology/biochemistry, virology, biodefense, emerging diseases, drug development and antibiotic resistance. Position Number 032324.

Biochemistry. Areas of interest include, but are not limited to, structure function relationships, protein and/or nucleic acid biochemistry, molecular basis of metabolic diseases and design/synthesis of therapeutics. Position Number 032323.

The Department is primarily housed in the six-story Biological Sciences Building that contains modern, spacious research laboratories and outstanding facilities for microscopic imaging, cell culture and nucleic acid analyses. Vertebrate and invertebrate animal facilities are also available. Wayne State University is a large, comprehensive, nationally ranked research institution that offers generous startup packages. Applicants must have a Ph.D. degree, postdoctoral experience, and an outstanding record of research achievement. Successful applicants are expected to establish and maintain vigorous, externally funded research programs and participate in graduate and undergraduate education. Applications will be considered only if they are received online at **website: http://jobs.wayne.edu.** Applicants must indicate the position(s) for which they are applying by marking the corresponding Position Number(s). In addition to their online applications that include curriculum vitae and cover letter, applicants must submit a statement of research plans along with a statement of teaching interests and philosophy, and have three letters of reference sent to: **Chair, Faculty Search Committee, Department of Biological Sciences, Wayne State University, 5047 Gullen Mall, Detroit, MI 48202.** Review of applications will begin immediately and the search will remain open until the positions have been filled. Applications will be considered only when all materials have been received. *Wayne State University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are especially encouraged to apply.*

FACULTY POSITION in endocrinology and metabolism. The University of California, San Diego (UCSD) School of Medicine, is actively recruiting a tenure track/tenured Junior Faculty Position in the Division of Endocrinology and Metabolism. Will consider either M.D. or M.D./Ph.D. candidates. Appointment level will be commensurate with experience and qualifications and compensation is based on established UCSD salary scales. Candidates should be able to develop an independent basic or clinical research program within the general field of endocrinology and metabolism. Clinical and teaching activities in the Division will be expected. Demonstrated productivity in basic or clinical research is required and current research support desirable but not mandatory. Must be board certified/eligible in endocrinology and metabolism and eligible for a state of California medical license. Review of applications will begin December 1, 2005, and will continue until position is filled. Apply to: **Jerrold M. Olefsky, M.D.,** sending application materials to the attention of: **Betsy Hansen, University of California, School of Medicine, Division of Endocrinology and Metabolism, 9500 Gilman Drive, MC-0673, La Jolla, CA 92093 0573.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR
Behavioral Neuropharmacology

The Department of Pharmacology, University of Tennessee (UT) Health Science Center, invites applications for a tenure track Assistant/Associate professor position in the area of behavioral neuropharmacology. We seek a faculty member who will be a major contributor to the UT Center of Excellence in the Neurobiology of Brain Diseases. The faculty member will direct an independent program in an aspect of psychopharmacology or drug abuse. Ideal candidates will be those who employ an integrative, mechanistic approach, utilizing neurochemical, molecular or electrophysiological techniques to complement their behavioral research. Candidates who will administer a core animal behavior laboratory will receive additional consideration. Candidates should be enthusiastic about engaging in collaborative research within a highly interactive community of neuroscientists. The selected candidate will participate in the teaching of graduate and professional students. Applicants should have a doctorate in pharmacology, neuroscience, experimental psychology or a related discipline, and relevant postdoctoral experience. Applications will be reviewed beginning December 1, 2005, but will continue to be accepted until the position is filled. Curriculum vitae, statement of research interests, and three reference letters should be sent to:

Jeffery D. Steketee, Ph.D., Behavioral Neuropharmacology Search Committee
Department of Pharmacology,
University of Tennessee Health Science Center
874 Union Avenue, Memphis, TN 38163
E-mail: jsteketee@utmem.edu.

The University of Tennessee is an Equal Opportunity / Affirmative Action / Title VI / Title IX / Section 504 / Americans With Disabilities Act / Age Discrimination in Employment Act Employer.

VERTEBRATE PHYSIOLOGIST: Lycoming College. The Department of Biology invites applications for a tenure-track **ASSISTANT PROFESSOR** for fall 2006. We seek an Animal Physiologist with expertise in terrestrial vertebrate studies. Teaching responsibilities include courses in human physiology, vertebrate zoology, a contribution to our introductory biology sequence, and an upper-level course in the area of expertise. Successful candidates must have a Ph.D. plus a strong commitment to teaching and involving undergraduates in research. Please visit the Department **website: http://www.lycoming.edu/biology/** for more information. Submit a letter of application, curriculum vitae, transcripts, a statement of teaching philosophy and a statement of research interests, and three letters of reference by December 15, 2005, to: **Dr. Michelle Briggs, Chair, Biology Department, Lycoming College, Campus Box 152, 700 College Place, Williamsport, PA 17701.** *Equal Opportunity Employer.*

The Interdisciplinary Department of Textile Engineering, Chemistry and Science at North Carolina State University invites applications for **TENURE-TRACK POSITIONS** in the areas of (1) fiber, polymer or materials engineering, and (2) the areas of modeling, simulation, and quality systems design. The positions are at the Assistant Professor level, but appropriate candidates may qualify for a named professorship. For a complete description of the vacant positions please see **website: http://www.tx.ncsu.edu/departments/tecs/positions.** Review of applications begins November 30, 2005, and continues until the positions are filled. *Affirmative Action/Equal Opportunity Employer. North Carolina State University welcomes all persons without regard to sexual orientation.*

POSITIONS OPEN

Fresno State, Department of Biology, is searching for two **ASSISTANT PROFESSOR POSITIONS.** For full consideration, all materials must be received by January 20, 2006.

(1) Cell Biologist, tenure-track. The successful applicant's expertise will be in signal transduction, cell cycle, cancer cell biology, developmental or stem cell biology, cellular motility, or membrane biology. The successful applicant is expected to establish an externally funded research program involving Master's and undergraduate students and to teach courses in our core and in his/her area of specialization. A Ph.D. in cell biology or a closely related field is required. Postdoctoral experience preferred. Send a letter of application (cover letter, online application form, curriculum vitae, statement of research interests and teaching philosophy, and three letters of reference), and confidential papers to: **Dr. Alejandro Calderón-Urrea, Department of Biology, California State University, Fresno, 2555 E. San Ramon Avenue M/S SB73, Fresno CA 93740,** or to **e mail: calalea@csufresno.edu. Telephone: 559 278 4080. Fax: 559 278 3963.**

(2) Vertebrate Physiologist/Neurobiologist, tenure-track. The successful applicant's expertise will be in any area of neuroscience, and will be expected to teach a course in neurophysiology, and a graduate course in their areas of specialty. The successful candidate must develop a research program that involves undergraduate and Master's level students, and pursue the external funding necessary to maintaining a successful research effort. An earned doctorate (Ph.D.) in biology, zoology, physiology or neurobiology is required for appointment to a tenure-track position. Postdoctoral experience preferred. Send a letter of application (cover letter, online application form, curriculum vitae, statement of research interest and teaching philosophy, and three letters of reference), and confidential papers to: **Dr. Brian Tsukimura (e mail: BrianT@CSUFresno.edu), Department of Biology M/S SB73, California State University, Fresno, 2555 E. San Ramon Avenue M/S SB73, Fresno, CA 93740. Telephone: 559-278-4244. Fax: 559-278 3963.**

California State University, Fresno, is an Equal Opportunity Employer.

ASSISTANT/ASSOCIATE PROFESSOR
Neuroscience, Department of Physiology
College of Medicine

We invite applications for a tenure-track position at the level of Assistant or Associate Professor. Candidates must have a Ph.D. or M.D. and postdoctoral experience. He or she must have demonstrated excellence in research in regulatory and integrative physiology with particular emphasis on neural systems or plasticity. The successful applicant will be expected to join the Neural Systems and Plasticity Research Group and to develop a strong externally funded research program. A competitive startup package is available. The successful candidate will be encouraged to submit an application to the Canadian Foundation of Innovation (CFI). She or he will contribute to the teaching of undergraduate students, within the proposed School of Biomedical Sciences and to the supervision of graduate students.

Please send curriculum vitae and the names of three references by January 15, 2006, to:

Dr. Wolfgang Walz
Head of Physiology
College of Medicine
University of Saskatchewan
107 Wiggins Road
Saskatoon SK S7N 5E5 Canada
E-mail: walz@sask.usask.ca
Fax: 306 966 6532

The University of Saskatchewan is committed to Employment Equity. Members of designated groups (women, Aboriginal people, people with disabilities, and visible minorities) are encouraged to self-identify on their applications. All qualified candidates are encouraged to apply, however, Canadians and permanent residents will be given priority.

THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

Professor and Chair of Nutritional Sciences

The Department of Nutritional Sciences of Rutgers University seeks a renowned scientist to chair and build the department to a level of international prominence. To make a significant impact on human health, Rutgers has targeted nutrition and its health consequences as a principal area for programmatic growth. The successful candidate will take a leadership role through the recruitment of new faculty, the development of major new facilities, and the fostering of multidisciplinary research and training programs. Nutritional Sciences is located at Cook College, Rutgers' school of food and environmental sciences and site of its Land Grant mission and activities, and there are ongoing collaborations with other Cook departments including Animal Sciences, Plant Biology and Pathology, Food Science, and Biotechnology. Nutritional Sciences is also part of a vibrant life sciences research community at Rutgers University, including major programs in structural biology, molecular, cellular and developmental biology, neuroscience, the Stem Cell Institute of New Jersey, the Cancer Institute of New Jersey, the Center for Advanced Biotechnology and Medicine, the Center of Alcohol Studies, the Environmental and Occupational Health Sciences Institute, the Human Genetics Institute, The School of Public Health, and the Robert Wood Johnson Medical School. The campus is located in central New Jersey, close to New York City, Philadelphia, beaches, and countryside.

The **Nutritional Sciences Department** has 17 full-time faculty members involved in undergraduate, graduate, and outreach programs. The faculty's research areas include lipid metabolism, calcium and bone development, energy expenditure and obesity, amino acid metabolism, child nutrition, community nutrition, and health promotion (**http://nutrition.rutgers.edu/**). The successful candidate will strengthen and extend the department's research areas in health and clinical fields involving the etiology, prevention, and treatment of nutrition-related diseases, including obesity, diabetes, CVD, cancer and osteoporosis. The successful candidate will direct the Nutritional Sciences Department, direct the department's research and educational programs, and oversee faculty mentoring.

Qualifications: The successful candidate must have a Ph.D. and/or M.D. or their equivalent and a record of distinguished research and scientific leadership. The successful candidate should have strong interpersonal skills and a sustained record of peer-reviewed publications and research funding. The successful candidate will be provided with a highly competitive salary, significant start-up support and laboratory space, and substantial administrative support. This is a tenure track position.

Inquiries and nominations should go to **Dr. Michael A. Gallo, Professor, Environmental and Occupational Medicine, Robert Wood Johnson Medical School, 170 Frelinghuysen Rd., Piscataway, NJ, 08854 (magallo@eohsi.rutgers.edu)**.

A letter of application, curriculum vitae, names of four or more professional references, and a statement of research and leadership objectives should be sent by electronic or regular mail to **Ms. Phyllis Lepucki, Rm 004, Martin Hall, 88 Lipman Drive, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901 (lepucki@aesop.rutgers.edu)**. A review of applications will begin on **February 15, 2006** and continue until a suitable candidate is identified. Starting date is negotiable, on or after July 1, 2006.

Rutgers University is an Equal Opportunity/Affirmative Action Employer.

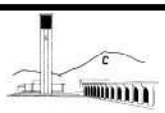
Assistant or Associate Professor of Neurobiology

Developmental Neurobiology Program
Institute of Molecular Medicine and Genetics
Medical College of Georgia

The Medical College of Georgia (MCG) invites applications for tenure-track Assistant or Associate Professor positions in the Program in Developmental Neurobiology, Institute of Molecular Medicine and Genetics. Candidates should have PhD or MD, postdoctoral experience, and potential to develop or maintain a strong extramurally funded research program in developmental or regenerative neurobiology. The MCG is a growing state supported academic medical center located in a historic city with outstanding recreational and lifestyle opportunities.

Interested applicants should submit a CV, a statement of research interests, and future plans, and should arrange for 3 letters of reference to: **Dr. Lin Mei, C/O Kathleen Murphree (kmurphree@meg.edu)**. Applications will be received until the position is filled. Please reference **ACII#'s 49384** and/or **49385** when applying. (PO# E0000075872)

W/V/F/D - EEO/AA



MULTIPLE FACULTY POSITIONS
UNIVERSITY OF CALIFORNIA, RIVERSIDE
BOURNS COLLEGE OF ENGINEERING

The Bourns College of Engineering at the University of California, Riverside invites applications for tenure-track or tenured faculty positions at the Assistant, Associate, or Professor Rank. The College is seeking highly qualified faculty members in the areas of Bioengineering, Chemical Engineering, Computer Engineering, Computer Science, Electrical Engineering, Environmental Engineering, Material Science and Engineering, and Mechanical Engineering. Specific areas of interest are provided at www.engr.ucr.edu/facultysearch/. People with vigorous research programs and demonstrated graduate student productivity are strongly encouraged to apply for the senior rank. Applicants should have a doctoral degree in the relevant engineering discipline or a related field; those with a bachelor's degree in engineering are preferred. Salary level will be competitive and commensurate with qualifications and experience.

We anticipate that the successful applicant will complement the highly motivated and entrepreneurial spirit of the College faculty, contributing meaningfully to the success of future teaching, research, and service accomplishments. Faculty research activities are essential to the success of our program and as such new members are expected to initiate and sustain strong sponsored research and graduate training programs.

The Bourns College of Engineering is proud of its faculty's accomplishments and rapid growth. Over the past five years, the numbers of faculty and undergraduates have nearly doubled; graduate student enrollment has increased six-fold, and research expenditures have more than tripled. The College currently has 70 faculty members, 1400 undergraduates, more than 300 graduate students, and more than $30 million in annual research expenditures. The College is home to five interdisciplinary and multidisciplinary research centers: The Center for Environmental Research and Technology (CE-CERT), the Center for Research in Intelligent Systems (CRIS), the Center for Nanoscale Science and Engineering (CNSE), the Center for Bioengineering, and the Network Embedded Computing Systems Institute (NECSI).

The College recently opened its Engineering II building as well as the Bourns Hall Clean Room facility (part of CNSE), and is expecting the opening of two additional buildings, Material Science and Engineering, and Engineering III in 2008 and 2011, respectively.

The search committee will begin reviewing applications on **January 1, 2006**, and will continue to receive applications until the positions are filled. To apply please register through the weblink at **www.engr.ucr.edu/facultysearch/** and submit the requested PDF or Word files (cover letter, curriculum vitae, statements of research and teaching interests, and reference contact information). For inquiries and questions, please contact us at **facultysearch@engr.ucr.edu**.

The University of California, Riverside is an Equal Opportunity/Affirmative Action Employer

POSITIONS OPEN

ASSOCIATE PROFESSOR / PROFESSOR
Cardiovascular Research

We are seeking an established investigator with outstanding research accomplishments to complement and extend the cardiovascular research strengths in our Department and our Institution. Medical College of Georgia has a strong cardiovascular research community with specific interests in nitric oxide, oxidative stress, eicosanoids, signal transduction, hypertension, endothelial dysfunction, angiogenesis, adhesion proteins, ion channels, myocardial disease, diabetes, and development of physical cardiovascular risk factors. We seek applicants with a strong record of productivity and an extramurally funded research program, particularly a program involved in control of vascular growth and cardiovascular dysfunctions. Physician scientists are encouraged to apply. We offer a generous startup package and outstanding core facilities are available for microarray technology, genetically modified animals, cell imaging, electron microscopy, primate research, and clinical collaborations. The successful applicant for this position will also participate in teaching programs for professional and graduate students. Please send curriculum vitae, summary of professional and research goals, and the names and addresses of three references to: **R. William Caldwell, Ph.D., Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912 2300. E mail: wcaldwel@mail.mcg.edu** and visit the Department homepage **website: http://www.mcg.edu/SOM/phmtox/index.html.** Application review will begin November 2005. *MCG is an Equal Employment Opportunity/Affirmative Action Equal Employment Opportunity/Affirmative Action Equal Access employer.*

PLANT ECOLOGIST

The W.K. Kellogg Biological Station (KBS) (**website: http://www.kbs.msu.edu**) of Michigan State University (MSU) seeks applicants for a tenure-track Assistant Professorship in plant ecology. We seek an interactive colleague in any area of plant ecology who will take advantage of the field and research facilities at KBS. Responsibilities will include development of an externally funded research program, teaching at KBS and on the main MSU campus in East Lansing, and participation in the graduate program in Ecology, Evolutionary Biology, and Behavior at MSU (**website: http://www.msu.edu/~eebb**). The successful candidate will be resident at KBS and have a joint appointment in the Department of Plant Biology (**website: http://www.plantbiology.msu.edu**) on the MSU campus.

Applicants should send curriculum vitae and statements of research and teaching interests, and arrange to have three letters of recommendation sent to: **Jeffrey Conner, Chair, Plant Ecologist Search Committee, W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060.** Address questions to **e-mail: connerj@msu.edu.** Review of applications will begin on 7 December 2005, and will continue until a suitable candidate is identified.

MSU is an Affirmative Action/Equal Opportunity Institution; women and minorities are particularly welcome to apply.

POSTDOCTORAL POSITION: Position available to study the pathophysiology of the septohippocampal system. The laboratory work is focused in understanding septal neuronal networks and their role in abnormal excitability states including Alzheimer's disease. The position requires expertise in electrophysiology. Skills in immunohistochemistry, tissue culture and molecular biology are desirable. Please send curriculum vitae, summary of research interests and three letters of reference to: **Luis V. Colom (e-mail: luis.colom@utb.edu), Department of Biological Sciences, The University of Texas at Brownsville, 80 Fort Brown, Brownsville, Texas 78520.**

POSITIONS OPEN

FACULTY POSITION
Visual Neuroscience

The Department of Vision Sciences and the Center for the Development of Functional Imaging at The University of Alabama at Birmingham (UAB) invite applications for a tenure-earning Faculty Position. Candidates with expertise in imaging (functional magnetic resonance imaging or optical) in experimental animals or humans are particularly encouraged to apply. UAB is one of America's premier research universities, ranking among the top 20 in funding from the National Institutes of Health. Vision research is especially strong at UAB with 52 faculty from eleven departments constituting the Vision Science Research Center, one of ten university-wide centers. The successful candidate will have access to MRI facilities which include two 3T human systems, a 9.4T small animal system, and a vertical 4.7T system dedicated to non human primate research. He/she will also have access to fully staffed core resources which include electronics, computer, machine shop, histology, molecular biology, transgenic, hybridoma, confocal microscopy, fluorescence resonance energy transfer (FRET), and multiphoton imaging. Vision Sciences faculty actively participate in many graduate programs including vision science, cellular and molecular biology, neuroscience, and biomedical engineering. For Assistant level appointment, candidates are expected to have doctoral and postdoctoral training, and to develop a nationally recognized and extramurally funded independent research program. Associate or Full Professor appointment will require demonstrated independence and research productivity, including a strong track record of external funding and peer reviewed publications. Applications should be received by January 31, 2006, to ensure full consideration. Applicants should send curriculum vitae including at least three references, and a statement of research interests to:

Dr. Paul Gamlin
Chair, Department of Vision Sciences
The University of Alabama at Birmingham
924 South 18th Street
Birmingham AL 35294
E-mail: pgamlin@uab.edu.

UAB is an Equal Opportunity Affirmative Action Employer.

IOWA STATE UNIVERSITY
Department of Physics and Astronomy
Physics of Biological Systems

Applications are invited for a tenure track **FACULTY** position to begin August 2006. We seek candidates with the strongest credentials and promise of future accomplishment in a forefront area of the physics of biological systems. The successful applicant will be expected to interact with researchers within the department as well as researchers in other disciplines. Potential approaches include single molecule studies, spectroscopy, diffraction methods and theoretical biological physics. Candidates at the assistant professor level are expected to have a Ph.D. in physics or a closely related discipline and a demonstrated record of research accomplishments normally achieved through postdoctoral experience. All candidates should demonstrate promise for excellence in teaching at both the undergraduate and graduate levels. Further information about the Physics and Astronomy Department and the life sciences program at ISU are on the web at **websites: http://www.physics.iastate.edu** and **http://www.bioinformatics.iastate.edu/.**

Applicants should send a letter of application, a resume including a statement of research and teaching interests, along with names, and contact information for at least three references. Please arrange for these letters of recommendation to be sent to: **Physics of Biological Systems Search Committee, c/o Ms. Gloria Oberender, Department of Physics and Astronomy, Iowa State University, Ames, Iowa, 50011 3160.** E mail applications will not be considered. Applications will be accepted until December 15, 2005, or until the position is filled. *Iowa State University is an equal opportunity employer. Women and minorities are encouraged to apply.*

POSITIONS OPEN

ASSOCIATE/FULL PROFESSOR POSITION
Tissue Engineering
The University of Texas, Arlington

The Bioengineering Department at The University of Texas, Arlington (UTA) invites applications for a senior level tenure (Associate or Full Professor) faculty position, with the starting employment date of fall 2006. Applicants must have a Ph.D. in bioengineering or a closely related field and a proven track of teaching, scholarly research, and external funding in tissue engineering or related areas such as biomaterials and biomechanics with tissue engineering focus. The Bioengineering Department has a joint M.S. and Ph.D. program with The University of Texas Southwestern Medical Center at Dallas, providing excellent opportunities for research collaboration with clinicians and life scientists. The successful candidate is expected to establish a vigorous, externally funded research program, teach graduate and undergraduate courses, and perform university service. UTA is ideally situated between the Dallas and Fort Worth metropolitan areas with less than 18 miles distance to both cities and excellent access to a large number of medical centers, high tech companies and cultural events. To apply, please visit our **website: https://cse2.uta.edu/be/te/** and upload your cover letter, curriculum vitae, statement of career goals, and names of at least three references. Review of applications will begin immediately and continue until the position is filled. *UTA is an Equal Opportunity, Affirmative Action Employer.*

VISITING FELLOW IN SCIENCE, TECHNOLOGY, AND ENVIRONMENTAL POLICY
Woodrow Wilson School
Princeton University

The Program in Science, Technology, and Environmental Policy (STEP) at the Woodrow Wilson School of Public and International Affairs, Princeton University, invites outstanding faculty, independent scholars, and practitioners to apply for an appointment as a fellow for the academic year 2006–2007. The successful candidate will devote an academic year in residence at Princeton to research, discussions, and scholarly collaboration on topics related to environmental policy and science. Under exceptional circumstances, applications for only one semester in residence may be considered. All applicants should have a doctorate or a professional postgraduate degree and at least several years of subsequent experience.

Full details regarding the fellowship and the application process can be found at **website: http://www.wws.princeton.edu/step/employmentframe.html.** The deadline for applications is December 22, 2005.

For more information about applying to Princeton please link to **website: http://web.princeton.edu/sites/dof/ApplicantsInfo.htm.**

Princeton University is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITIONS are available to examine the role of Mdm2 and Mdm4 in regulation of the p53 tumor suppressor during development, cell differentiation, and oncogenesis using cell culture and mouse models. In addition, successful candidates will have the opportunity to train in all aspects of mouse modeling. Experience in molecular biology is required.

Send curriculum vitae and contact information for three references to: **Stephen Jones, Ph.D., Department of Cell Biology, UMass Medical School, 55 Lake Avenue North, Worcester, MA 01655. E-mail: stephen.jones@umassmed.edu.** Visit website: **http:// www.umassmed.edu/cellbio/faculty/jones.cfm.**

POSITIONS OPEN

PIONEER
A DUPONT COMPANY

RESEARCH SCIENTIST

Pioneer Hi-Bred International, Incorporated is the world leader in the discovery, development and delivery of elite crop genetics. We are looking for a Research Scientist at our Johnston, Iowa location to provide technical and scientific expertise supporting crop protection gene expression studies in fungi and plants. Ph.D. in biological sciences and a minimum of seven years of research experience (post Ph.D.) or equivalent combination of education and experience. Extensive personal experience in the quantitative analysis of gene expression. Direct experience with analysis of RNA using quantitative methodologies including microarrays and real time PCR is required. Excellent written and spoken communication skills and experience with manipulation of large data sets are required. The ability to contribute at the technical, scientific and management levels is required.

Required identification for this position is TP460. For a complete job description and to apply, go to **website: http://www.pioneer.com/employment.** *Equal Opportunity Employer.*

POSTDOCTORAL FELLOW
Pathology and Laboratory Medicine
Emory University School of Medicine

Postdoctoral positions are available at Emory University focusing on innate immunity, epithelial cell biology and the pathophysiology of epithelial inflammation. Opportunities exist for involvement in exciting projects aimed at understanding how leukocytes interact with epithelial cells with special emphasis on cell-cell adhesion/integrins, signaling, transmigration and epithelial barrier function. The role of junctional adhesion molecules and signal regulatory proteins in the above processes are actively being studied. A doctoral degree, experience in molecular/cell biology and protein biochemistry of eukaryotic systems, and strong English communication skills are required. Preference will be given to individuals with previous experience in the fields of innate immunity and/or biology of epithelial cells. The successful applicant will join our epithelial pathobiology group comprised of six principal investigators with common interests that occupy 12,500 square feet of new, fully equipped and interconnected research space. Interested individuals should send resume to: **Dr. Charles Parkos, Emory University, Whitehead Biomedical Research Building, Room 105B, 615 Michael Street Atlanta GA 30322. E-mail: cparkos@emory.edu.**

Emory University is an Equal Opportunity/Affirmative Action Employer.

IMMUNOLOGIST, TENURE TRACK

The Department of Cellular Biology at the University of Georgia invites applicants for a tenure-track Assistant Professor position in the area of immunology. This position is part of a major expansion of programs in biomedical sciences, in conjunction with the opening of the Paul D. Coverdell Center for Interdisciplinary Biomedical Studies. Successful candidates will be expected to develop a strong extramurally funded research program and to contribute to instruction in immunology. Individuals whose research interests complement our institutional and departmental foci in infectious diseases, developmental biology or cell biology are of particular interest. Potential applicants can learn more about the programs in this highly collaborative environment and find details about the application process at **website: http://immunology.cb.uga.edu.** Applications received by January 15, 2006, are assured of full consideration. *The Franklin College of Arts and Sciences is highly committed to increasing the diversity of its faculty and strongly encourages applications from members of underrepresented groups. The University of Georgia is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

THE UNIVERSITY OF TEXAS
Southwestern Medical Center

ASSISTANT/ASSOCIATE PROFESSOR

POSITION: The Department of Physiology invites scientists with a track record of technical and intellectual innovation to apply for a tenure-track Assistant or Associate Professor position. M.D., Ph.D., or equivalent degree is required. This position is targeted to individuals who can bring new technologies to fruition to answer important physiological or systems biological questions. Optical, mechanical, electrical, molecular biological, or computational methods are all appropriate with important applications expected at one or more levels of physiology, ranging from individual genes and proteins to cells and organs.

This position represents a new growth phase of this Department at one of the world's leading medical science centers. The position will be supported by significant laboratory space on our new campus, a competitive salary and an exceptional startup package. University of Texas (UT) Southwestern is the scientific home of four Nobel Prize laureates and 15 members of the National Academy of Sciences. More than 2,000 research projects are supported by $300 million grant funding annually at our school.

Applicants should submit electronically a curriculum vitae and brief statement of research plans to **e-mail: donald hilgemann search@utsouthwestern.edu** and should arrange to have three confidential letters of reference forwarded to the same address.

UT Southwestern strongly encourages applications from women, minorities, and people with disabilities. An Affirmative Action/Equal Opportunity Employer.

FACULTY POSITION IN VIROLOGY
University of Hawai'i

The Department of Microbiology at the University of Hawai'i at Manoa invites applications for a tenure track, nine month faculty position in virology at the Assistant or Associate Professor level. The expected start date is August 1, 2006. The Department seeks an individual using modern molecular approaches in the area of animal virology. Candidates must have a Ph.D. in the biological sciences, postdoctoral research experience, a record of publications and a commitment to teaching. A minimum of four years full time teaching experience at the Assistant Professor level is required for application at the Associate level. The successful candidate will be expected to develop an independent, externally funded research program, to teach at the undergraduate and graduate levels, and to mentor students. Salary will be commensurate with experience and rank. Competitive startup package to be offered. Applicants should send curriculum vitae, statements of teaching philosophy and research interests, and the names and contact information of three references to: **Virologist Search Chair, Department of Microbiology, University of Hawai'i, 207 Snyder Hall, 2538 McCarthy Mall, Honolulu, HI 96822.** Inquiries should be directed to: **Dr. Sean Callahan** at **e-mail: scallaha@hawaii.edu.** Applications received after January 1, 2006, may not receive full consideration. *The University of Hawai'i is an Equal Opportunity/Affirmative Action Institution.*

ASSISTANT PROFESSOR POSITION
ASSOCIATE RESEARCH SCIENTIST
Division of Hematology/Oncology
Maimonides Medical Center, Brooklyn, NY
Mount Sinai School of Medicine

We are seeking an Associate Research Scientist to continue the ongoing research of myelofibrosis. Applicant must have a U.S. Ph.D. degree and expertise in molecular biology. Salary ranges from $45,000 to $55,000 annually plus fringe benefits commensurate with experience. Send your curriculum vitae directly to: **Jen C. Wang, M.D., Maimonides Medical Center Brooklyn, New York, Division of Hematology/Oncology; e mail: jcwang5@aol.com; fax: 718-635-7110.**

POSITIONS OPEN

Northeastern UNIVERSITY BOSTON, MA

ASSISTANT OR ASSOCIATE PROFESSOR
The Barnett Institute of
Chemical and Biological Analysis

The Barnett Institute of Chemical and Biological Analysis announces two new positions at the Assistant or Associate Professor level. These appointments are part of a $75 million investment plan launched by Northeastern University, and will be held jointly between the Barnett Institute and the Department of Chemistry and Chemical Biology. Joint appointments with other departments in the Colleges of Arts and Sciences, Engineering, or Pharmaceutical Sciences will also be considered.

The Institute, which recently celebrated its thirtieth anniversary as a pioneer in the application of emerging bioanalytical approaches to address contemporary biological and clinical challenges, seeks outstanding candidates who can complement its current research programs in proteomics, metabolomics and associated technologies for systems biology (**website: http://www.barnett.neu.edu**). Major collaborations are ongoing with nearby medical schools as well as biotechnology and pharmaceutical companies, and the institute has an active technology licensing program. Relevant fields of research could include carbohydrate chemistry, nanotechnology, clinical diagnostics or bioengineering; other areas may also be of interest. Assistant Professor candidates (tenure track) should have at least two years of postdoctoral experience. Associate Professor candidates (tenured) should have a demonstrated level of accomplishment, including an active, federally funded research program. Please send a letter of application and a comprehensive curriculum vitae to: **Dr. Roger Kautz; Barnett Institute, Room 341 Mugar Building; Northeastern University, 360 Huntington Avenue; Boston, MA 02115.** Or by **e-mail: barnettinst@neu.edu.**

Northeastern University is an Equal Opportunity/Affirmative Action Employer, and actively encourages applications from women and minorities.

Leonard M. Miller School of Medicine
University of Miami
Department of Dermatology and Cutaneous Surgery

The Leonard M. Miller School of Medicine, University of Miami, Department of Dermatology and Cutaneous Surgery is seeking a full time faculty member at the **RESEARCH ASSISTANT PROFESSOR / RESEARCH ASSOCIATE PROFESSOR / RESEARCH PROFESSOR** level. Candidates must have M.D., Ph.D., or M.D./Ph.D. We are seeking individuals with research interests in dermatology, inflammatory infections, genetic diseases, and/or wound healing expertise desirable. Over 1,000 square feet of wet laboratory space. Staff, Ph.D., and fellow support to be negotiated. External grant support is desirable. In addition, clinical responsibilities for M.D. or M.D./Ph.D. applicants to include seeing private patients at current clinical practice for general dermatology. Rank and salary will be commensurate with training and experience. Please forward curriculum vitae to: **Lawrence A. Schachner, M.D., Chairman & Harvey Blank Professor, P.O. Box 016250, Miami, FL 33101. E-mail: lschachn@med.miami.edu. Telephone: 305 243 4771. Fax: 305 243 6191.** *An Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOWSHIP

Texas A&M University at Galveston (TAMUG) seeks applications for two competitive two year Postdoctoral Fellowships from highly qualified candidates interested in any aspect of marine biology, oceanography, coastal/ocean engineering, marine geology, or marine policy and management. For details, see **website: http://www.tamug.edu/postdoc.** *Equal Opportunity Employer/Affirmative Action.*

POSITIONS OPEN

FACULTY POSITION
Department of Biological Sciences
Purdue University

The Department of Biological Sciences invites applications for a tenure-track faculty position in vertebrate developmental biology. Primary consideration will be given to candidates who use zebrafish or mice as genetic animal models to ask fundamental questions in developmental biology. Technical expertise in high-throughput approaches for screening or gene knockdowns, microRNAs, or transgenic knockouts is desirable. We expect to fill an academic year appointment at the **ASSISTANT PROFESSOR** level; however, appointment at a higher rank will be considered for qualified applicants.

The Department has over 50 faculty members directing research in a wide range of fields from bioinformatics, through molecular and systems levels, to evolutionary biology and ecology. Over the next several years we anticipate additional faculty positions in developmental biology, integrative disease biology, and molecular evolution. The Department directs a transgenic mouse core facility in conjunction with the Purdue Cancer Center and maintains an animal facility. Further information about the Department is available at **website: http://www.bio.purdue.edu/.** The University is expanding the life sciences on campus and as part of this initiative several new buildings are nearing completion. These include a new Biomedical Engineering Building, a Structural Biology Building, and the Bindley Bioscience Center, which houses shared facilities for image analysis, genomics, quantitative and functional proteomics, and other biological instrumentation.

The successful vertebrate developmental biology applicant must have a Ph.D. or equivalent in an appropriate discipline and at least two years of postdoctoral experience. We seek applicants with a strong potential for excellence in research, the promise of extramural funding, and a commitment to excellence in teaching. Applications must be submitted electronically as a PDF file that includes a detailed curriculum vitae, the names and addresses of three references, a summary of research interests, and a one-paragraph teaching statement to **e-mail: chair_devo@bio.purdue.edu.** Inquiries should be directed to: **Professor Donna M. Fekete, Chair of Developmental Search Committee, Department of Biological Sciences, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054.** Review of applications has begun and will continue until the position is filled.

The Department also plans to fill, in a college-wide effort called COALESCE, a number of other biology faculty positions in multidisciplinary areas, including membrane science, bioinformatics, and nanoscience. Applicants in these fields may apply directly to **website: http://www.science.purdue.edu/COALESCE/.** Applicants to one search may be included in other relevant searches when appropriate.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer and is committed to building a diverse faculty of excellence.

BENTHIC ECOLOGIST: Career-track position, GS-12 entry level, is available at the U.S. Geological Survey (USGS), Florida Integrated Science Center to conduct targeted field and laboratory marine and estuarine community research. Applicants must have a strong background in field oriented quantitative marine benthic invertebrate ecology. Applicants must have a strong record of independent research, external funding, and publication. Previous postdoctoral experience is essential. The successful candidate will assume a lead role in ongoing multi disciplinary deep-water research, and develop a high-caliber research program, sustained largely on external funding. Direct inquiries to: **Dr. Kenneth Sulak (e mail: ken_sulak@usgs.gov).** Applicants will find details, and must apply online at: **website: http://www.usgs.gov/oht/oars/.** Vacancy number ER-S-2005-0070. Opens 10 November 2005, closes 9 December 2005. *USGS is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

FACULTY POSITION
MEDICAL MICROBIOLOGIST

The Biological Sciences Department at California State Polytechnic (Cal Poly) University, Pomona, invites applications for a tenure-track Assistant Professor position, beginning September 2006. Candidates must have a strong commitment to excellence in teaching and research. The candidate should be able to teach both traditional and molecular diagnostic techniques to microbiology, medical technology and biotechnology majors. Teaching responsibilities will include lower division and upper division/graduate microbiology courses, such as medical bacteriology and medical parasitology, and other specialty courses, as well as participation in introductory biology courses. The successful candidate is expected to develop an extramurally funded research program involving undergraduate and Master's level students. Ph.D. is required; teaching and postdoctoral experience is preferred. Cal Poly Pomona is a comprehensive Master's level university with a diverse student body. The successful candidate will have demonstrated ability to be responsive to the educational equity goals of the university and its increasing ethnic diversity and international character. Applicants should send: (1) curriculum vitae, (2) statement of teaching philosophy, (3) proposed plan of research, (4) representative publication reprints, and (5) the names and contact information of five references to: **Dr. Jill Adler Moore, Chair, Medical Microbiologist Search Committee, Biological Sciences Department, California State Polytechnic University, 3801 West Temple Avenue, Pomona, CA 91768 4132 (e mail: jpadler@csupomona.edu).** Review of applications will begin December 18, 2005, and will continue until position is filled. Official transcripts and three letters of reference will be required of all finalists. For further information, visit the Department **website: http://www.csupomona.edu/~biology.**

California State Polytechnic University, Pomona is an Equal Opportunity, Affirmative Action Employer. Cal Poly Pomona subscribes to all state and federal regulations and prohibits discrimination based on gender, race, sexual orientation, national origin, disability, marital status, age, religion, or veteran status.

FACULTY POSITION
Department of Chemistry and Biochemistry
Center for Protein Structure and Function
University of Arkansas

The Department of Chemistry and Biochemistry at the University of Arkansas is seeking an outstanding scientist for a tenure track faculty position associated with the NIH National Center for Research Resources Center for Protein Structure and Function. **(website: http://www.uark.edu/chemistry).** Research areas appropriate for the position include nuclear magnetic resonance (NMR) determination of protein structure and dynamics, drug design, bioorganic chemistry, and spectroscopic studies of proteins. The Center has five new NMR spectrometers, including a 700 MHz NMR and a 500 MHz NMR, both with cryoprobes. State-of-the-art protein X ray crystallography and mass spectrometry core facilities are also associated with the Center. Collaborative, multidisciplinary research projects are encouraged. Successful candidates must have a Ph.D. and postdoctoral experience and will be expected to establish a nationally funded research program, and teach effectively at the graduate and undergraduate levels. Review of completed applications will begin on December 15, 2005, and continue until the position is filled. Curriculum vitae, description of research interests, and three letters of recommendation should be sent to: **Professor Frank Millett, Chair, Faculty Search Committee, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701 (e-mail: millett@uark.edu).** *Women and minority candidates who will contribute to the diversity of the campus community are especially encouraged to apply. The University of Arkansas is an Affirmative Action/Equal Opportunity Employer and applications will be accepted without regard to age, race, color, sex, or national origin. Applicants must have proof of legal authority to work in the United States.*

POSITIONS OPEN

FACULTY POSITION
Department of Biological Sciences
Purdue University

The Department of Biological Sciences invites applications for a tenure-track faculty position in microbial pathogenesis. We wish to identify candidates who focus on host-pathogen interactions using an animal model of infectious disease. Primary consideration will be given to candidates who use mice as an animal model to study fundamental questions in bacterial or viral pathogenesis. We expect to fill an academic year appointment at the **ASSISTANT PROFESSOR** level; however, appointment at a higher rank will be considered for qualified applicants.

The Department has over 50 faculty members directing research in a wide range of fields from bioinformatics, through molecular and systems levels, to evolutionary biology and ecology. Over the next several years we anticipate additional faculty positions in developmental biology, integrative disease biology, and molecular evolution. The Department directs a transgenic mouse core facility in conjunction with the Purdue Cancer Center and maintains an animal facility. Further information about the Department is available at **website: http://www.bio.purdue.edu/.** The University is expanding the life sciences on campus and as part of this initiative several new buildings are nearing completion. These include a new Biomedical Engineering Building, a Structural Biology Building, and the Bindley Bioscience Center, which houses shared facilities for image analysis, genomics, quantitative and functional proteomics, and other biological instrumentation.

The successful microbial pathogenesis applicant must have an M.D., Ph.D., or equivalent in an appropriate discipline and at least two years of postdoctoral experience. We seek applicants with a strong potential for excellence in research, the promise of extramural funding, and a commitment to excellence in teaching. Applications must be submitted electronically as a PDF file that includes a detailed curriculum vitae, the names and addresses of three references, a summary of research interests, and a one-paragraph teaching statement to e-mail: **chair_micro@bio.purdue.edu.** Inquiries should be directed to: **Professor Allan E. Konopka, Chair of Microbial Pathogenesis Search Committee, Department of Biological Sciences, Purdue University, 915 W. State Street, West Lafayette, IN 47907-2054.** Review of applications will begin November 30, 2005, and continue until the position is filled.

The Department also plans to fill, in a college wide effort called COALESCE, a number of other biology faculty positions in multidisciplinary areas, including membrane science, bioinformatics, and nanoscience. Applicants in these fields may apply directly to **website: http://www.science.purdue.edu/COALESCE/.** Applicants to one search may be included in other relevant searches when appropriate.

Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer and is committed to building a diverse faculty of excellence.

POSTDOCTORAL POSITION

A Postdoctoral position is available in proteomics at Texas Tech University, Lubbock, Texas. This position involves studies on development of methodologies for quantitative analyses of proteins in proteomics research. The applicant must be familiar with proteomics and must have strong expertise in protein separation by 1-D and 2-D electrophoresis as well as in handling MALDI mass spectrometry and peptide mass fingerprinting. The applicant should also have excellent communication and writing skills, and must be able to conduct research independently. Applicants should send curriculum vitae including the names and contact addresses of at least three references to: **Dr. Satomi Niwayama, e-mail: satomi.niwayama@ttu.edu.**

University of California Riverside

TITLE/RANK: Professor, Associate Professor, or Assistant Professor. Appointment rank and salary commensurate with experience.

LOCATION: University of California, Riverside, California.

POSITION: The College of Natural and Agricultural Sciences invites applications for two faculty positions in stem cell biology beginning on or after July 1, 2006. We are particularly interested in individuals studying the differentiation of human embryonic stem cells and their potential application to human therapy, although all areas of mammalian embryonic stem cell research will be considered. The successful candidates will interact with our interdisciplinary stem cell focus group consisting of faculty from the life sciences, engineering, and biomedical sciences. The stem cell faculty will also be part of our developing Health Sciences Research Institute. Highly competitive start-up packages and state-of-the-art facilities are available. UC Riverside is a rapidly growing campus with central proximity to the major biomedical research areas in Southern California.

QUALIFICATIONS: Applicants must hold a Ph.D., M.D. or equivalent and have postdoctoral experience. Candidates must have demonstrated expertise in stem cell biology.

RESPONSIBILITIES: The successful candidates will establish and maintain vigorous, innovative research programs in stem cell biology taking advantage of new state funding opportunities, as well as federal and private sources. Opportunities for graduate student training are available through interdepartmental graduate programs in Neuroscience; Cell Molecular and Developmental Biology; Biomedical Sciences; Biochemistry and Molecular Biology; Genetics, Genomics and Bioinformatics, and a developing program in Bioengineering. Teaching responsibilities would be at the graduate and undergraduate levels.

TO APPLY: Applications should contain a curriculum vitae, brief statement of research interests, relevant reprints, and the names, addresses, phone and fax numbers, and email addresses of three references. Applications can be submitted electronically to **stemcells@ucr.edu**. Alternatively, hardcopy applications can be submitted to **Chair, Stem Cell Search Committee, 1208 Spieth Hall, University of California, Riverside, CA, 92521**.

DEADLINE: Review of applications will begin **December 11, 2005** and will continue until the positions are filled.

The University of California is an Equal Opportunity/Affirmative Action Employer.

University of Oxford

Department of Chemistry

Inorganic Chemistry Laboratory Postdoctoral Research Assistant

Grade RS1A for University Research staff, salary £22,289 - £24,352 p.a.

There is a vacancy for a Postdoctoral Research Assistant to work with Dr Jason Davis. This PDRA position, renewable for three years, concerns the construction of novel, anion-selective luminescent rotaxanes and catenanes, including surface-assembled systems. The post requires experience of fluorescence/luminescence and surface assembly. The successful applicant will already have a PhD or have submitted a thesis prior to taking up the appointment. The post is for one year in the first instance.

Further particulars are available from the Administrator, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR (quoting reference DH05020/JJD), or by e-mail (rita.higgs@chem.ox.ac.uk) and these must be obtained before application is made.

Informal enquiries may be made to Dr Jason Davis (e-mail: jason.davis@chem.ox.ac.uk).

Four copies of applications in the form of a letter, curriculum vitae and the names and addresses of two academic referees, at least one of whom should be your current 'line manager' or supervisor, who may be contacted prior to interview, showing how you fulfil the selection criteria, should be sent (hard copy only), quoting the reference number to: The Administrator, (reference DH05020/JJD) Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, by the closing date is 1st December 2005.

The University is an Equal Opportunities Employer.

Yale University School of Medicine
Interdepartmental Program
Cellular Neuroscience, Neurodegeneration, and Repair
333 Cedar Street
New Haven, CT 06510

Faculty Positions

Yale University is establishing a Program for Cellular Neuroscience, Neurodegeneration, and Repair to bring together scientists involved in basic and translational neuroscience research. Aims of the Program are to:
(a) understand neuron-specific aspects of cell function,
(b) elucidate the cellular pathophysiology of neurodegeneration and
(c) translate this knowledge into therapies capable of repairing the nervous system and improving neuronal function in disease.
The Program will emphasize biophysical, molecular and genetic approaches and foster interactions across disciplinary boundaries. Faculty, who will be appointed to existing academic departments, will be housed in common research space at the School of Medicine.

Seven new faculty members will be appointed over the next several years. Candidates must hold an M.D. and/or a Ph.D. degree, or equivalent degrees. We invite applications at the rank of assistant professor, but appointments at the rank of associate and full professor will be considered. The first round of applications is due by December 31, 2005. Please send a cover letter, curriculum vitae, up to 3 representative publications, a research plan (strictly limited to 2 pages), and arrange for submission of 3 letters of recommendation.

Application materials should be sent electronically to **Pietro De Camilli** and **Stephen M. Strittmatter**, co-directors of the Program, exclusively at the following e-mail address: **cnnr.search@yale.edu**. Recommendation letters can be forwarded by mail.

Applications from, or nominations of, women and minority scientists are encouraged. Yale is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

GENOMICS FACULTY POSITION

The Department of Biology at the University of South Florida (USF) announces a tenure track position at the **ASSISTANT PROFESSOR** level beginning August 2006. Research interests should be in the general area of genomics. Candidates that can interact with our dynamic group of faculty with strengths in cell and molecular biology, conservation biology and marine biology are encouraged to apply. Candidates must have a Ph.D. in one of the biological sciences, postdoctoral experience and relevant publications. The successful candidate will be expected to develop an active, externally funded research program, and teach an undergraduate course in genetics and graduate courses in their area of specialization. Send curriculum vitae, reprints of three published papers, statements of research and teaching interests, and three letters of reference to: **The Genomics Search Committee, Department of Biology, University of South Florida, 4202 E. Fowler Avenue, SCA 110, Tampa, Florida 33620 5200.** Complete applications, including letters, must be received by December 15, 2005. According to Florida Law, applications and meetings regarding them are open to the public. *For ADA accommodations, please contact* ***Dawn McGowan*** *at* ***telephone: 813-974-3250*** *at least five working days prior to need. USF is an Affirmative Action/Equal Employment Opportunity institution.*

POSTDOCTORAL FELLOWS
University of Cincinnati Department of Molecular Genetics, Biochemistry and Microbiology

Postdoctoral positions are available in the area of Na, K-ATPase function and physiology. Using gene targeting, we have developed models to investigate the individual roles for each of the alpha isoforms of this enzyme. Both standard and conditional knockouts and gene replacements have been produced. This represents a great opportunity for individuals seeking experience related to physiology of ion transport regulation or training in organ systems such as heart, muscle, vascular system, kidney, ovary, et cetera. The studies involve a multidisciplinary approach including but not limited to physiology and biochemistry. The training environment within the department is outstanding with 27 faculty, 45 graduate students, and approximately equal number of postdoctoral fellows. Interested candidates should send their resumes to: **Jerry B. Lingrel, Ph.D., Professor and Chair, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, College of Medicine, 231 Albert Sabin Way, Cincinnati, Ohio 45267-0524** or e-mail: **Jerry.Lingrel@uc.edu.**

POSTDOCTORAL POSITIONS
Hormone Receptor Signaling/Breast Cancer Northwestern University

Positions are available in a molecular endocrinology laboratory focused on the function of the prolactin receptor complex during the pathogenesis of breast cancer. Research areas include the action of the Nek3/Vav2 complex in proximal receptor signaling, the pathophysiologic function of the six recognized human prolactin receptor isoforms, contribution of prolactin receptor phosphorylation to receptor function, and the regulation of Stat signaling by co regulators and cyclophilin B. Molecular biology, cell culture, and/or rodent husbandry experience required. Cutting edge methodologies employed by the laboratory include, but are not limited to, analysis using yeast two-hybrid and transcriptional array analysis; proteomics/mass spectroscopy; and xenograft and knockout models of breast cancer. Recent graduates with U.S. citizenship or permanent residence are encouraged to apply. Please send curriculum vitae, a statement of research interests, and three references via e mail to: **Dr. Charles Clevenger, Department of Pathology and Breast Cancer Research Program, Northwestern University** at e mail: **clevenger@northwestern.edu.** *Equal Opportunity Employer/Affirmative Action.*

POSITIONS OPEN

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POSTDOCTORAL RESEARCH POSITION

The Biology Department at Amherst College seeks a Postdoctoral Fellow interested in collaborative research and teaching within the college environment. Research may be conducted within the laboratory of any member of the Department (**website: http://www.amherst.edu/~biology/faculty.html**) but must address some aspect of genomic biology. Participation in the teaching of undergraduates will involve the co-teaching of a course, or a specific module within a course, with faculty in the Department. The two year position is funded by a grant from the Howard Hughes Medical Institute to Amherst College to support teaching and research in the area of genomic biology. Review of applications will begin after January 1, 2006, and continue until the position is filled. More details of the position and the application process are available at **website: http://www.amherst.edu/~biology/menu.html.**

Amherst College is a private undergraduate liberal arts college for men and women, with 1,600 students and 190 faculty members. Located in the Connecticut River Valley of western Massachusetts, Amherst participates with Hampshire, Mount Holyoke, and Smith Colleges and the University of Massachusetts in the Five-College Consortium. *Amherst College is an Equal Opportunity/Affirmative Action Employer, and encourages women, persons of color and persons with disabilities to apply. The administration, faculty and staff are committed to attracting candidates from groups currently underrepresented on our campus.*

POSTDOCTORAL POSITION: Calcium Signaling in Smooth Muscle.

Postdoctoral Position immediately available to study calcium sparks, calcium waves, and potassium channels in arterial smooth muscle cells. Experience with cardiovascular physiology, patch-clamp electrophysiology, confocal microscopy and/or calcium imaging preferred. Required qualifications include a Ph.D. or M.D. in physiology or a related field. Send curriculum vitae and names and addresses of three references to:

Jonathan H. Jaggar, Ph.D.
Department of Physiology
University of Tennessee Health Science Center
894 Union Avenue
Memphis, TN 38163 U.S.A.
E-mail: jjaggar@physio1.utmem.edu.

The University of Tennessee is an Equal Employment Opportunity /Affirmative Action/Title VI/Title IX/Section 504/ADA/ADEA Employer.

The Center for Aquatic Ecology and Conservation at the Illinois Natural History Survey seeks a **POSTDOCTORAL ASSOCIATE** to participate in research on the potential impact of invasive filter feeding Asian carp in the Great Lakes. Ph.D. in aquatic ecology/limnology required; research experience in plankton or fisheries biology preferred. For the full position announcement and application instructions see **website: http://www.inhs.uiuc.edu/opportunities/index.html.** Apply by January 6, 2006, for full consideration. For technical questions, contact: **Dr. Walter Hill (e mail: wrhill@uiuc.edu, telephone: 217-244-2103)** or **Dr. Robert Herendeen (e mail: herendee@uiuc.edu, telephone: 217-244-2137).**

POSITIONS OPEN

The Virginia Maryland Regional College of Veterinary Medicine has an immediate opening for a **POSTDOCTORAL ASSOCIATE** to perform molecular and microbiological investigations on specimens obtained from wild chimpanzees. Various molecular and microbiological techniques will be applied to study wild chimpanzee populations as it relates to their health, well-being and conservation. Emphasis will be on microsatellite analysis. The incumbent will assist with the training of graduate students on their laboratory studies involving molecular and microbiological techniques, and assist with the preparation of grant proposals. Candidates must have a Ph.D. with demonstrated experience with both microbiological and molecular techniques, including, microsatellite analysis, DNA isolation, PCR, cloning, and culturing and isolating microbes. Candidates with a Ph.D., that also have a D.V.M., V. M. D. or M. D. are preferred. Evidence of appropriate research training with a record of publications in peer-reviewed journals is required. Demonstrated skill in scientific writing for peer reviewed journal publications and/or grant proposals is required. Candidates must be able to design and execute laboratory experiments, analyze data and prepare manuscripts for publication. Candidates must be self-motivated, capable of working independently, and able to discern when it is important to confer with the principal investigator. A willingness to work with graduate students is required.

Submit application online at **website: https://jobs.vt.edu** and upload supporting documents, including: cover letter, curriculum vitae, academic background, research experience and publications, and three letters of recommendation by November 06, 2005.

Funding is guaranteed for three years.

Additional information may be obtained from:

Dr. Taranjit Kaur
Virginia Tech CRC XV (0493)
Blacksburg, VA 24061
Telephone: 540-231-6522. Fax: 540-231-7735
E-mail: taranjit@vt.edu

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POSTDOCTORAL POSITION immediately available to develop novel therapeutic modalities for the treatment of graft versus host disease (GVHD) in the context of allogeneic hematopoietic stem cell transplantation by targeting inflammatory signaling pathways. Requirements: M.D. or Ph.D. degree in immunology, cell biology, or molecular biology. Strong background in murine models, molecular biologic methodologies and flow cytometry, is highly desirable. curriculum vitae with names, telephone numbers, and e mail addresses of three references should be sent to: **Markus Y. Mapara, M.D., Ph.D., Division of Hematology-Oncology University of Pittsburgh Cancer Institute, e mail: maparamy@upmc.edu. Telephone: 412-623-1112.**

NIH-funded **POSTDOCTORAL POSITION** to explore enzyme catalytic mechanisms using newly developed transient state kinetic approaches (*Accounts of Chemical Research* **38**:157, 2005). Experience in protein structure function relationships, kinetic isotope effects, physical-organic chemistry, or some related area is desirable. Contact: **Harvey F. Fisher, Professor of Biochemistry, University of Kansas School of Medicine at: VA Medical Center, 4801 E. Linwood Boulevard, Kansas City, MO 64128 or telephone: 816 861 4700 extension 57156; fax: 816-861-1110;** or **e-mail: hfisher@kumc.edu.**

ASSISTANT CURATOR of Birds and Mammals and **POSTDOCTORAL RESEARCH ASSOCIATE.** We seek an Assistant Curator for the University of Missouri's Museum of Zoology. Primary responsibilities are collection curation, teaching one course per year, and research. For details see: **website: http://www.snr.missouri.edu/fw/.** Submit application materials to: **M.E. Gompper, Department of Fisheries and Wildlife Sciences, University of Missouri, Columbia, MO 65211.** Review of applications begins December 15, 2005.

GRANTS

Research Grants

Inflammatory Bowel Diseases

- Seeking exploratory proposals for innovative research in IBD (Crohn's disease and ulcerative colitis).
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Telephone: 1-310-954-5091

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POSITIONS OPEN

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Carnegie Mellon QATAR CAMPUS
Five Anniversary
مؤسسة قطر Qatar Foundation

Human-Computer Interaction Visiting Faculty Position in the School of Computer Science

Carnegie Mellon University established a branch campus in Qatar in the fall of 2004. We are offering a BS degree in Computer Science to an international student body. The university invites applications for a visiting faculty position to begin as early as January 2006.

We are seeking a faculty member in the area of Learning Science and Technology with research experience ideally in designing, implementing, deploying, and evaluating educational technology in school or college settings. An ability to teach courses in human–computer interaction, artificial intelligence, cognitive psychology, or related areas is also desired. The position will involve research in collaboration with the Pittsburgh Science of Learning Center and faculty at the Human-Computer Interaction Institute at Carnegie Mellon in Pittsburgh. The position offers competitive salaries, overseas assignments, travel and housing allowances and other benefits packages, as well as attractive research support.

Interested candidates should send their resume, statement of teaching interest and research, and names of three references to: **Faculty Hiring Committee, c/o Ruth Gaus, Qatar Office SMC 1070, 5032 Forbes Avenue, Pittsburgh, PA 15289; Ruth.Gaus@cs.cmu.edu; Fax 412-253-0924.**

- For more information on the Pittsburgh Science of Learning Center, see **http://learnlab.org**.
- For more information on the Human-Computer Interaction Institute, see **http://www.hcii.cs.cmu.edu**.
- For more information on the BS in CS program, see **http://www.csd.cs.cmu.edu/education/hsces/index.html**.
- For more information on the Carnegie Mellon Qatar Campus, see **http://www.qatar.cmu.edu/**.
- Information on Qatar is available at: **http://www.experienceqatar.com/**.

MU

Assistant or Associate Professor BIOMEDICAL ENGINEERING

The department of Biological Engineering and the Dalton Cardiovascular Research Center at the University of Missouri – Columbia invite applications for a tenure-track or tenured faculty position. Candidates with research strengths related to cell membrane physiology and/or bioMEMS are preferred. The successful candidate will also teach at the undergraduate and graduate levels. Competitive salary, start-up package and laboratory facilities will be provided.

MU offers a rich environment for collaboration and Columbia is consistently ranked as one of the top 20 places to live in the U.S. The Biological Engineering department is rapidly expanding with faculty expertise in biosensors, bioMEMS, biomaterials, electrophysiology, biomechanics, and biophotonics. The Membrane Physiology Group at the Dalton Center consists of 9 faculty from 4 colleges who study membrane-associated transport processes such as ion channel gating, transporter function, and exocytosis of neurotransmitter.

Applicants should have an earned doctoral degree in biomedical engineering or a related field and a strong background in both engineering and life sciences. Senior-level candidates are expected to have a vigorous, extramurally funded research program whereas candidates applying at the Assistant Professor level must have a high potential for establishing an externally funded research program. Postdoctoral training is preferred. Review of applications will begin on **December 1, 2005** and will continue until the position is filled.

Applicants should submit a Curriculum Vitae, a summary of past research and future research plans, a brief statement of teaching plans, and a list of three to five professional references to: **Search Committee Chair, Dept. of Biological Engineering, 215 Ag Eng. Bldg., University of Missouri, Columbia, MO 65211. Ph: (573) 882-2369**, Email: **RatliffDe@missouri.edu**.

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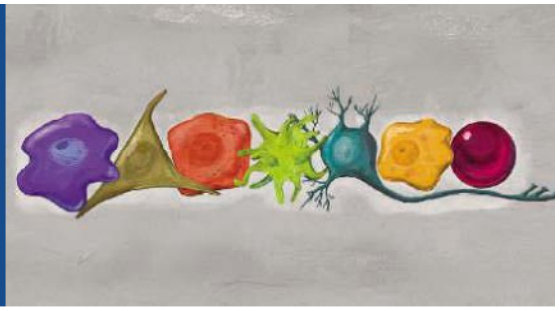

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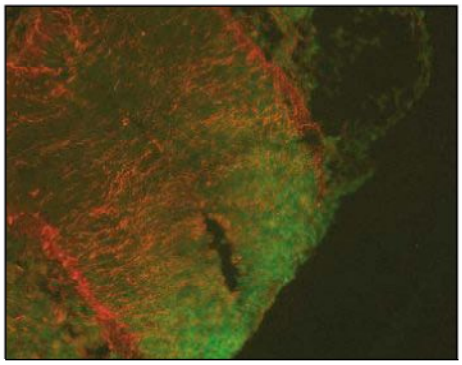

FIGURE 1. Detection of Nestin in mouse fetal brain tissues using R&D Systems' goat anti-rat Nestin polyclonal antibody (Catalog # AF2736). Sections were stained using Rhodamine Red-conjugated anti-goat secondary antibody and counterstained using Fluoro Nissl Green.

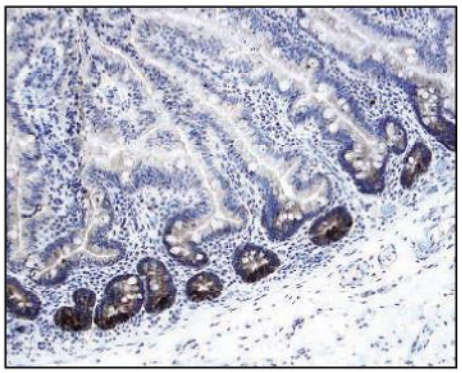

FIGURE 2. Detection of Musashi-1 in paraffin-embedded human intestine tissue sections using 10 mg/mL of R&D Systems' goat anti-human affinity-purified antibody (Cat. # AF2628). Tissues were stained using R&D Systems' anti-goat HRP-DAB Cell and Tissue Staining Kit (brown) (Cat. # CTS008) and counterstained with hematoxylin (blue). Tissue antigen retrieval was done using R&D Systems' antigen retrieval reagent (basic pH, Cat. # CTS013)

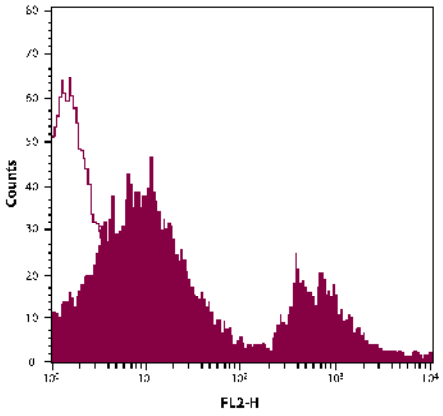

FIGURE 3. Intracellular staining of mouse embryonic stem cells differentiated by 5 µM retinoic acid for 3 days with anti-Oct3/4-PE (Catalog # IC1759P) (filled histogram) or with isotype control (Catalog # IC013P, open histogram).

R&D Systems cell marker antibodies facilitate stem cell characterization and assessment of differentiation.

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